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CONTENTS—PART 2

*7,809 volunteer abstracts, 18 symposium and workshop abstracts.

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CHRONOLOGICAL LIST OF SESSIONS

(see page xii for Thematic List of Sessions)

TUESDAY

THURSDAY

Thematic List of Sessions

(Includes slide and poster sessions, symposia, and workshops only.)

Theme A: Development and Plasticity

Session Number	Session Title	Type	Day and Time
509.	Aging: physiology	Poster	Fri AM
167.	Biochemical and pharmacological correlates of development I	Poster	Tue AM
258.	Biochemical and pharmacological correlates of development II	Poster	Wed AM
518.	Biochemical and pharmacological correlates of development III	Poster	Fri AM
132.	Cell lineage and determination I	Slide	Tue AM
359.	Cell lineage and determination II	Slide	Thu AM
453.	Cell lineage and determination III	Poster	Thu PM
416.	**Development of Functional Heterogeneity Among Sensory Neurons**	**Symp.**	**Thu PM**
39.	Differentiation and development I	Poster	Mon AM
69.	Differentiation and development II	Slide	Mon PM
171.	Differentiation and development III	Poster	Tue AM
291.	Differentiation and development IV	Poster	Wed AM
311.	Differentiation and development V	Slide	Wed PM
407.	Differentiation and development VI	Poster	Thu AM
414.	Differentiation and development VII	Poster	Thu AM
483.	Differentiation and development VIII	Slide	Fri AM
510.	Differentiation and development IX	Poster	Fri AM
112.	Endocrine control and development I	Poster	Mon PM
113.	Endocrine control and development II	Poster	Mon PM
282.	Endocrine control and development III	Poster	Wed AM
478.	Endocrine control and development IV	Slide	Fri AM
354.	Limbic system I	Poster	Wed PM
401.	Motor systems I	Poster	Thu AM
458.	Motor systems II	Poster	Thu PM
12.	Neural plasticity in adult animals: LTP I	Slide	Mon AM
225.	Neural plasticity in adult animals: LTP II	Poster	Tue PM
454.	Neural plasticity in adult animals: anatomy and behavior	Poster	Thu PM
245.	Neural plasticity in adult animals: central	Slide	Wed AM
338.	Neural plasticity in adult animals: cerebral cortex	Poster	Wed PM
197.	Neural plasticity in adult animals: induced effects I	Poster	Tue PM
334.	Neural plasticity in adult animals: induced effects II	Poster	Wed PM
277.	Neural plasticity in adult animals: neuromuscular and autonomic	Poster	Wed AM
487.	Neural plasticity in adult animals: peripheral	Slide	Fri AM
150.	Neuronal death I	Poster	Tue AM
449.	Neuronal death II	Poster	Thu PM
290.	Neurotoxicity in development I	Poster	Wed AM
352.	Neurotoxicity in development II	Poster	Wed PM
507.	Neurotoxicity: studies in tissue culture	Poster	Fri AM
122.	**New Approaches to the Functional Development of the Neocortex**	**Wksh.**	**Tue AM**
235.	Nutritional and perinatal factors in development	Poster	Tue PM
108.	Process outgrowth, growth cones, and guidance mechanisms I	Poster	Mon PM
184.	Process outgrowth, growth cones, and guidance mechanisms II	Slide	Tue PM
231.	Process outgrowth, growth cones, and guidance mechanisms III	Poster	Tue PM
240.	Process outgrowth, growth cones, and guidance mechanisms IV	Slide	Wed AM
300.	Process outgrowth, growth cones, and guidance mechanisms V	Slide	Wed PM
346.	Process outgrowth, growth cones, and guidance mechanisms VI	Poster	Wed PM

Theme B: Cell Biology

372.	Membrane composition and cell surface macromolecules I	Slide	Thu AM
406.	Membrane composition and cell surface macromolecules II	Poster	Thu AM
508.	Membrane composition and cell surface macromolecules III	Poster	Fri AM
11.	Messenger RNA regulation I	Slide	Mon AM
302.	Messenger RNA regulation II	Slide	Wed PM
350.	Messenger RNA regulation III	Poster	Wed PM
463.	Messenger RNA regulation IV	Poster	Thu PM
529.	Messenger RNA regulation V	Poster	Fri AM
62.	**Molecular Biological Approaches to Study Neuropeptide Biosynthesis**	**Symp.**	**Mon PM**
173.	Neuroglia: biology of astrocytes I	Poster	Tue AM
425.	Neuroglia: biology of astrocytes II	Slide	Thu PM
317.	Neuroglia: myelin forming cells	Poster	Wed PM
48.	Neuroglia: myelin mutants	Poster	Mon AM
219.	Staining and tracing techniques I	Poster	Tue PM
220.	Staining and tracing techniques II	Poster	Tue PM

Theme C: Excitable Membranes and Synaptic Transmission

Session Number	Session Title	Type	Day and Time
121.	**At Last! Potassium Channels: Expression and Regulation**	**Symp.**	**Tue AM**
54.	Ion channels: calcium channels I	Poster	Mon AM
55.	Ion channels: calcium channels II	Poster	Mon AM
56.	Ion channels: calcium channels III	Poster	Mon AM
363.	Ion channels: calcium channels IV	Slide	Thu AM
117.	Ion channels: cell function I	Poster	Mon PM
118.	Ion channels: cell function II	Poster	Mon PM
261.	Ion channels: chloride and other	Poster	Wed AM
260.	Ion channels: ligand-gated I	Poster	Wed AM
419.	Ion channels: ligand-gated II	Slide	Thu PM
57.	Ion channels: modulation and regulation I	Poster	Mon AM
64.	Ion channels: modulation and regulation II	Slide	Mon PM
262.	Ion channels: modulation and regulation III	Poster	Wed AM
303.	Ion channels: modulation and regulation IV	Slide	Wed PM
438.	Ion channels: modulation and regulation V	Poster	Thu PM
484.	Ion channels: potassium, chloride and other	Slide	Fri AM
241.	Ion channels: sodium channels I	Slide	Wed AM
335.	Ion channels: sodium channels II	Poster	Wed PM
358.	**Non-Uniformity of Synaptic Physiology and Implications for Plasticity in the Nervous System**	**Wksh.**	**Thu AM**
207.	Pharmacology of synaptic transmission I	Poster	Tue PM
325.	Pharmacology of synaptic transmission II	Poster	Wed PM
110.	Postsynaptic mechanisms I	Poster	Mon PM
111.	Postsynaptic mechanisms II	Poster	Mon PM
369.	Postsynaptic mechanisms III	Slide	Thu AM
186.	Potassium channels I	Slide	Tue PM
382.	Potassium channels II	Poster	Thu AM
439.	Presynaptic mechanisms I	Poster	Thu PM
486.	Presynaptic mechanisms II	Slide	Fri AM
31.	Presynaptic mechanisms: ions	Poster	Mon AM
440.	Presynaptic mechanisms: modulators	Poster	Thu PM
30.	Presynaptic mechanisms: toxins	Poster	Mon AM
44.	Synaptic structure and function I	Poster	Mon AM
252.	Synaptic structure and function II	Slide	Wed AM

Theme D: Neurotransmitters, Modulators, and Receptors

Session Number	Session Title	Type	Day and Time
432.	Acetylcholine	Poster	Thu PM
257.	Acetylcholine: basal forebrain and brainstem	Poster	Wed AM
530.	Acetylcholine: receptors and choline uptake	Poster	Fri AM
523.	Adrenergic receptor modulation and regulation	Poster	Fri AM
166.	Adrenergic receptors I	Poster	Tue AM
374.	Adrenergic receptors II	Poster	Thu AM
63.	**Afferent Regulation of the Locus Coeruleus**	**Wksh.**	**Mon PM**
71.	Amino acids: GABA and benzodiazepines I	Slide	Mon PM
143.	Amino acids: GABA and benzodiazepines II	Poster	Tue AM
144.	Amino acids: GABA and benzodiazepines III	Poster	Tue AM
183.	Amino acids: GABA and benzodiazepines IV	Slide	Tue PM
326.	Amino acids: GABA and benzodiazepines V	Poster	Wed PM
393.	Amino acids: GABA and benzodiazepines VI	Poster	Thu AM
125.	Behavioral pharmacology	Slide	Tue AM
455.	Behavioral pharmacology: acetylcholine	Poster	Thu PM
340.	Behavioral pharmacology: dopamine	Poster	Wed PM
506.	Behavioral pharmacology: miscellaneous	Poster	Fri AM
404.	Behavioral pharmacology: monoamines	Poster	Thu AM
91.	Behavioral pharmacology: psychostimulants	Poster	Mon PM
16.	Catecholamines I	Slide	Mon AM
163.	Catecholamines II	Poster	Tue AM
293.	Catecholamines III	Poster	Wed AM
376.	Catecholamines IV	Poster	Thu AM
433.	Catecholamines V	Poster	Thu PM
490.	Catecholamines VI	Poster	Fri AM
164.	Catecholamines: electrophysiology I	Poster	Tue AM
377.	Catecholamines: electrophysiology II	Poster	Thu AM
294.	Catecholamines: *in vivo* measurements	Poster	Wed AM
242.	Characterization of cholinergic receptors	Slide	Wed AM
94.	Characterization of muscarinic receptors I	Poster	Mon PM
435.	Characterization of muscarinic receptors II	Poster	Thu PM
95.	Characterization of nicotinic receptors	Poster	Mon PM
107.	Cholinergic receptor modulation and regulation	Poster	Mon PM
375.	Dopamine receptor modulation and regulation	Poster	Thu AM
165.	Dopamine receptors I	Poster	Tue AM
185.	Dopamine receptors II	Slide	Tue PM
378.	Dopamine receptors III	Poster	Thu AM
434.	Dopamine receptors IV	Poster	Thu PM
40.	Excitatory amino acids I	Poster	Mon AM
97.	Excitatory amino acids II	Poster	Mon PM
98.	Excitatory amino acids III	Poster	Mon PM
198.	Excitatory amino acids IV	Poster	Tue PM
199.	Excitatory amino acids V	Poster	Tue PM
318.	Excitatory amino acids VI	Poster	Wed PM
380.	Excitatory amino acids VII	Poster	Thu AM
381.	Excitatory amino acids VIII	Poster	Thu AM
421.	Excitatory amino acids IX	Slide	Thu PM
480.	Excitatory amino acids X	Slide	Fri AM
168.	Excitatory amino acids: excitotoxicity I	Poster	Tue AM
169.	Excitatory amino acids: excitotoxicity II	Poster	Tue AM
299.	Excitatory amino acids: excitotoxicity III	Slide	Wed PM
46.	Interactions between neurotransmitters I	Poster	Mon AM
348.	Interactions between neurotransmitters II	Poster	Wed PM
489.	Interactions between neurotransmitters III	Slide	Fri AM
2.	**Interleukin-1: Immune and Neural Modulator**	**Symp.**	**Mon AM**
182.	**Neuronal Gene Expression: Physiological Activation, Transcriptional Control and DNA Binding Proteins**	**Symp.**	**Tue PM**
218.	Opiates, endorphins and enkephalins: anatomy and chemistry I	Poster	Tue PM
280.	Opiates, endorphins and enkephalins: anatomy and chemistry II	Poster	Wed AM

18.	Opiates, endorphins and enkephalins: physiological effects I	Poster	Mon AM
162.	Opiates, endorphins and enkephalins: physiological effects II	Poster	Tue AM
191.	Opiates, endorphins and enkephalins: physiological effects III	Slide	Tue PM
461.	Opiates, endorphins and enkephalins: physiological effects IV	Poster	Thu PM
146.	Peptides: anatomical localization I	Poster	Tue AM
271.	Peptides: anatomical localization II	Poster	Wed AM
395.	Peptides: anatomical localization III	Poster	Thu AM
13.	Peptides: biosynthesis, metabolism and biochemical characterization I	Slide	Mon AM
161.	Peptides: biosynthesis, metabolism and biochemical characterization II	Poster	Tue AM
349.	Peptides: biosynthesis, metabolism and biochemical characterization III	Poster	Wed PM
462.	Peptides: biosynthesis, metabolism and biochemical characterization IV	Poster	Thu PM
58.	Peptides: physiological effects I	Poster	Mon AM
59.	Peptides: physiological effects II	Poster	Mon AM
60.	Peptides: physiological effects III	Poster	Mon AM
370.	Peptides: physiological effects IV	Slide	Thu AM
10.	Peptides: receptors I	Slide	Mon AM
145.	Peptides: receptors II	Poster	Tue AM
270.	Peptides: receptors III	Poster	Wed AM
394.	Peptides: receptors IV	Poster	Thu AM
45.	Receptor modulation and regulation I	Poster	Mon AM
229.	Receptor modulation and regulation II	Poster	Tue PM
423.	Receptor modulation and regulation III	Slide	Thu PM
43.	Regional localization of receptors and transmitters I	Poster	Mon AM
73.	Regional localization of receptors and transmitters II	Slide	Mon PM
314.	Regional localization of receptors and transmitters III	Poster	Wed PM
315.	Regional localization of receptors and transmitters IV	Poster	Wed PM
35.	Second messengers I	Poster	Mon AM
36.	Second messengers II	Poster	Mon AM
37.	Second messengers III	Poster	Mon AM
52.	Second messengers IV	Poster	Mon AM
53.	Second messengers V	Poster	Mon AM
175.	Second messengers VI	Poster	Tue AM
485.	Second messengers VII	Slide	Fri AM
129.	Serotonin receptors I	Slide	Tue AM
247.	Serotonin receptors II	Slide	Wed AM
15.	Serotonin, histamine and other biogenic amines I	Slide	Mon AM
88.	Serotonin, histamine and other biogenic amines II	Poster	Mon PM
89.	Serotonin, histamine and other biogenic amines III	Poster	Mon PM
221.	Serotonin, histamine and other biogenic amines IV	Poster	Tue PM
222.	Serotonin, histamine and other biogenic amines V	Poster	Tue PM
339.	Serotonin, histamine and other biogenic amines VI	Poster	Wed PM
99.	Transmitter uptake, storage, secretion and metabolism I	Poster	Mon PM
275.	Transmitter uptake, storage, secretion and metabolism II	Poster	Wed AM
479.	Transmitter uptake, storage, secretion and metabolism III	Slide	Fri AM
17.	Transmitters in invertebrates I	Slide	Mon AM
155.	Transmitters in invertebrates II	Poster	Tue AM
216.	Transmitters in invertebrates III	Poster	Tue PM
76.	Transmitters in invertebrates: gastropods	Slide	Mon PM
365.	Transmitters: acetylcholine	Slide	Thu AM

Theme E: Endocrine and Autonomic Regulation

Session Number	Session Title	Type	Day and Time
14.	Cardiovascular regulation I	Slide	Mon AM
82.	Cardiovascular regulation II	Poster	Mon PM
136.	Cardiovascular regulation III	Slide	Tue AM
206.	Cardiovascular regulation IV	Poster	Tue PM

250.	Cardiovascular regulation V	Slide	Wed AM
391.	Cardiovascular regulation VI	Poster	Thu AM
392.	Cardiovascular regulation VII	Poster	Thu AM
422.	Hypothalamic-pituitary-adrenal regulation I	Slide	Thu PM
514.	Hypothalamic-pituitary-adrenal regulation II	Poster	Fri AM
515.	Hypothalamic-pituitary-adrenal regulation III	Poster	Fri AM
176.	Hypothalamic-pituitary-gonadal regulation I	Poster	Tue AM
177.	Hypothalamic-pituitary-gonadal regulation II	Poster	Tue AM
431.	Hypothalamic-pituitary-gonadal regulation III	Slide	Thu PM
528.	Neural control of adrenal function	Poster	Fri AM
93.	Neural control of immune system I	Poster	Mon PM
304.	Neural control of immune system II	Slide	Wed PM
512.	Neural control of immune system III	Poster	Fri AM
513.	Neural control of immune system IV	Poster	Fri AM
209.	Neuroendocrine controls: other I	Poster	Tue PM
426.	Neuroendocrine controls: other II	Slide	Thu PM
469.	Neuroendocrine controls: other III	Poster	Thu PM
33.	Neuroendocrine controls: pituitary I	Poster	Mon AM
126.	Neuroendocrine controls: pituitary II	Slide	Tue AM
256.	Neuroendocrine controls: pituitary III	Poster	Wed AM
130.	Regulation of autonomic function I	Slide	Tue AM
217.	Regulation of autonomic function II	Poster	Tue PM
527.	Regulation of autonomic function III	Poster	Fri AM
189.	Respiratory regulation I	Slide	Tue PM
255.	Respiratory regulation II	Poster	Wed AM
379.	Respiratory regulation III	Poster	Thu AM
297.	**Specificity in the Control of Regional Sympathetic Outflow: Physiological, Neurochemical and Anatomical Approaches**	**Symp.**	**Wed PM**

Theme F: Sensory Systems

Session Number	Session Title	Type	Day and Time
473.	Chemical senses: gustatory pathways	Poster	Thu PM
474.	Chemical senses: olfactory pathways	Poster	Thu PM
428.	Chemical senses: peripheral mechanisms I	Slide	Thu PM
466.	Chemical senses: peripheral mechanisms II	Poster	Thu PM
296.	**Coding of Visual Signals at the Photoreceptor Synapse**	**Wksh.**	**Wed PM**
476.	**Form and Synaptic Function in Retinal Ganglion Cells**	**Symp.**	**Fri AM**
3.	**Functional Properties of Somatic Sensory "Barrel Field" Cortex**	**Wksh.**	**Mon AM**
153.	Invertebrate sensory systems I	Poster	Tue AM
154.	Invertebrate sensory systems II	Poster	Tue AM
341.	Pain modulation: biogenic amines	Poster	Wed PM
75.	Pain modulation: central pathways I	Slide	Mon PM
342.	Pain modulation: central pathways II	Poster	Wed PM
284.	Pain modulation: opioid mechanisms	Poster	Wed AM
134.	Pain modulation: pharmacology	Slide	Tue AM
283.	Pain modulation: stress and sensory stimulation	Poster	Wed AM
368.	Pain pathways	Slide	Thu AM
49.	Pain pathways: central mechanisms	Poster	Mon AM
224.	Pain pathways: inflammation, sensitization and hyperalgesia	Poster	Tue PM
223.	Pain pathways: long-term changes	Poster	Tue PM
464.	Pain pathways: trigeminal system	Poster	Thu PM
19.	Retina I	Poster	Mon AM
68.	Retina II	Slide	Mon PM
147.	Retina III	Poster	Tue AM
244.	Retina IV	Slide	Wed AM
396.	Retina V	Poster	Thu AM
450.	Retina VI	Poster	Thu PM
133.	Sensory systems: auditory systems I	Slide	Tue AM
200.	Sensory systems: auditory systems II	Poster	Tue PM
201.	Sensory systems: auditory systems III	Poster	Tue PM

263.	Sensory systems: auditory systems IV	Poster	Wed AM
264.	Sensory systems: auditory systems V	Poster	Wed AM
321.	Sensory systems: auditory systems VI	Poster	Wed PM
441.	Sensory systems: auditory systems VII	Poster	Thu PM
442.	Sensory systems: auditory systems VIII	Poster	Thu PM
289.	Somatic and visceral afferents	Poster	Wed AM
159.	Somatic and visceral afferents: dorsal root ganglion cells	Poster	Tue AM
285.	Somatic and visceral afferents: somesthetic afferents	Poster	Wed AM
92.	Somatosensory cortex I	Poster	Mon PM
286.	Somatosensory cortex II	Poster	Wed AM
306.	Somatosensory cortex III	Slide	Wed PM
279.	Spinal cord	Poster	Wed AM
50.	Subcortical somatosensory pathways: brainstem and thalamus	Poster	Mon AM
465.	Subcortical somatosensory pathways: trigeminal system	Poster	Thu PM
20.	Subcortical visual pathways I	Poster	Mon AM
127.	Subcortical visual pathways II	Slide	Tue AM
333.	Subcortical visual pathways III	Poster	Wed PM
397.	Subcortical visual pathways IV	Poster	Thu AM
8.	Visual cortex I	Slide	Mon AM
85.	Visual cortex II	Poster	Mon PM
187.	Visual cortex III	Slide	Tue PM
243.	Visual cortex IV	Slide	Wed AM
362.	Visual cortex V	Slide	Thu AM
451.	Visual cortex VI	Poster	Thu PM
502.	Visual cortex VII	Poster	Fri AM

Theme G: Motor Systems and Sensorimotor Integration

Session Number	Session Title	Type	Day and Time
34.	Basal ganglia and thalamus: motor systems I	Poster	Mon AM
66.	Basal ganglia and thalamus: motor systems II	Slide	Mon PM
287.	Basal ganglia and thalamus: motor systems III	Poster	Wed AM
408.	Basal ganglia and thalamus: motor systems IV	Poster	Thu AM
409.	Basal ganglia and thalamus: motor systems V	Poster	Thu AM
430.	Basal ganglia and thalamus: motor systems VI	Slide	Thu PM
417.	**Coding and Execution of Movement in Three Dimensions**	**Symp.**	**Thu PM**
400.	Invertebrate motor function	Poster	Thu AM
202.	Motor systems and sensorimotor integration: cerebellum I	Poster	Tue PM
305.	Motor systems and sensorimotor integration: cerebellum II	Slide	Wed PM
497.	Motor systems and sensorimotor integration: cerebellum III	Poster	Fri AM
9.	Motor systems and sensorimotor integration: circuitry and pattern generation I	Slide	Mon AM
104.	Motor systems and sensorimotor integration: circuitry and pattern generation II	Poster	Mon PM
67.	Motor systems and sensorimotor integration: cortex I	Slide	Mon PM
142.	Motor systems and sensorimotor integration: cortex II	Poster	Tue AM
208.	Motor systems and sensorimotor integration: cortex III	Poster	Tue PM
274.	Motor systems and sensorimotor integration: cortex IV	Poster	Wed AM
329.	Motor systems and sensorimotor integration: cortex V	Poster	Wed PM
248.	Motor systems and sensorimotor integration: oculomotor system I	Slide	Wed AM
320.	Motor systems and sensorimotor integration: oculomotor system II	Poster	Wed PM
386.	Motor systems and sensorimotor integration: oculomotor system III	Poster	Thu AM
387.	Motor systems and sensorimotor integration: oculomotor system IV	Poster	Thu AM
28.	Motor systems and sensorimotor integration: posture and movement I	Poster	Mon AM
29.	Motor systems and sensorimotor integration: posture and movement II	Poster	Mon AM

105.	Motor systems and sensorimotor integration: posture and movement III	Poster	Mon PM
106.	Motor systems and sensorimotor integration: posture and movement IV	Poster	Mon PM
192.	Motor systems and sensorimotor integration: posture and movement V	Slide	Tue PM
384.	Motor systems and sensorimotor integration: posture and movement VI	Poster	Thu AM
385.	Motor systems and sensorimotor integration: posture and movement VII	Poster	Thu AM
496.	Motor systems and sensorimotor integration: posture and movement VIII	Poster	Fri AM
74.	Motor systems and sensorimotor integration: vestibular system I	Slide	Mon PM
137.	Motor systems and sensorimotor integration: vestibular system II	Poster	Tue AM
138.	Motor systems and sensorimotor integration: vestibular system III	Poster	Tue AM
383.	Muscle: function and biochemistry	Poster	Thu AM
495.	Muscle: structural characteristics	Poster	Fri AM
319.	Reflex function: general	Poster	Wed PM
521.	Reflex function: human I	Poster	Fri AM
522.	Reflex function: human II	Poster	Fri AM
427.	Spinal cord and brainstem	Slide	Thu PM
140.	Spinal cord and brainstem: anatomy	Poster	Tue AM
78.	Spinal cord and brainstem: electrophysiology	Poster	Mon PM
139.	Spinal cord and brainstem: immunocytochemistry	Poster	Tue AM
32.	Spinal cord and brainstem: lesion studies	Poster	Mon AM
79.	Spinal cord and brainstem: motor output	Poster	Mon PM

Theme H: Other Systems of the CNS

Session Number	Session Title	Type	Day and Time
328.	Association cortex and thalamocortical relations	Poster	Wed PM
472.	Brainstem systems	Poster	Thu PM
23.	Cerebral metabolism and blood flow I	Poster	Mon AM
371.	Cerebral metabolism and blood flow II	Slide	Thu AM
399.	Cerebral metabolism and blood flow III	Poster	Thu AM
488.	Cerebral metabolism and blood flow IV	Slide	Fri AM
26.	Comparative neuroanatomy: amphibians, reptiles, birds	Poster	Mon AM
504.	Comparative neuroanatomy: cerebral cortex	Poster	Fri AM
25.	Comparative neuroanatomy: fish	Poster	Mon AM
51.	Hippocampus and amygdala I	Poster	Mon AM
100.	Hippocampus and amygdala II	Poster	Mon PM
343.	Hippocampus and amygdala III	Poster	Wed PM
470.	Hypothalamus I	Poster	Thu PM
471.	Hypothalamus II	Poster	Thu PM
373.	Limbic system II	Poster	Thu AM
181.	**New Insights into the Functions of the Basal Forebrain Cholinergic System**	**Symp.**	**Tue PM**

Theme I: Neural Basis of Behavior

Session Number	Session Title	Type	Day and Time
21.	Alcohol I	Poster	Mon AM
83.	Alcohol II	Poster	Mon PM
281.	Alcohol III	Poster	Wed AM

477.	**Neuropeptides, Steroids and Behavior**	**Symp.**	**Fri AM**
22.	Psychotherapeutic drugs I	Poster	Mon AM
87.	Psychotherapeutic drugs II	Poster	Mon PM
151.	Psychotherapeutic drugs III	Poster	Tue AM
239.	**Sex Differences and Hormonal Influences on Cognitive Brain Function**	**Symp.**	**Wed AM**
178.	Stress, hormones and autonomic nervous system I	Poster	Tue AM
179.	Stress, hormones and autonomic nervous system II	Poster	Tue AM

Theme J: Disorders of the Nervous System

Session Number	Session Title	Type	Day and Time
42.	Alzheimer's disease I	Poster	Mon AM
65.	Alzheimer's disease II	Slide	Mon PM
259.	Alzheimer's disease: amyloid	Poster	Wed AM
437.	Alzheimer's disease: neuropathology	Poster	Thu PM
361.	Alzheimer's disease: protein	Slide	Thu AM
493.	Alzheimer's disease: transmitters	Poster	Fri AM
505.	Behavioral disorders	Poster	Fri AM
80.	Cerebral ischemia I	Poster	Mon PM
205.	Cerebral ischemia II	Poster	Tue PM
327.	Cerebral ischemia III	Poster	Wed PM
398.	Cerebral ischemia IV	Poster	Thu AM
429.	Cerebral ischemia V	Slide	Thu PM
503.	Cerebral ischemia VI	Poster	Fri AM
141.	Clinical CNS neurophysiology I	Poster	Tue AM
312.	Clinical CNS neurophysiology II	Poster	Wed PM
7.	Degenerative disease: Parkinson's	Slide	Mon AM
525.	Degenerative disease: Parkinson's (nonprimates)	Poster	Fri AM
157.	Degenerative disease: Parkinson's (primates)	Poster	Tue AM
420.	Degenerative disease: other I	Slide	Thu PM
526.	Degenerative disease: other II	Poster	Fri AM
468.	Developmental disorders	Poster	Thu PM
6.	Epilepsy I	Slide	Mon AM
102.	Epilepsy II	Poster	Mon PM
194.	Epilepsy III	Slide	Tue PM
345.	Epilepsy IV	Poster	Wed PM
412.	Epilepsy: GABA and benzodiazepines	Poster	Thu AM
227.	Epilepsy: hippocampus and neocortex I	Poster	Tue PM
351.	Epilepsy: hippocampus and neocortex II	Poster	Wed PM
411.	Epilepsy: peptides	Poster	Thu AM
228.	Epilepsy: second messengers and mRNA	Poster	Tue PM
459.	Epilepsy: substantia nigra and amygdala	Poster	Thu PM
195.	Genetic models I	Slide	Tue PM
332.	Genetic models II	Poster	Wed PM
520.	Infectious diseases	Poster	Fri AM
273.	Neuromuscular diseases	Poster	Wed AM
135.	Neurotoxicity I	Slide	Tue AM
313.	Neurotoxicity II	Poster	Wed PM
436.	Neurotoxicity III	Poster	Thu PM
491.	Neurotoxicity IV	Poster	Fri AM
492.	Neurotoxicity V	Poster	Fri AM
357.	**New Genes from Old Diseases**	**Symp.**	**Thu AM**
253.	Trauma I	Slide	Wed AM
460.	Trauma II	Poster	Thu PM

314.17

ONTOGENY OF MUSCARINIC M2 RECEPTORS IN THE RAT BRAIN — AN AUTORADIOGRAPHIC STUDY OF ^{3}H-AF DX 116 BINDING SITES. R. Miyoshi, S. Kito and S. Yamashita*. Third Dept. of Internal Med., Hiroshima Univ. School of Med., Hiroshima 734, Japan.

Previously, we examined the ontogeny of muscarinic acetylcholine receptors in the rat brain through in vitro autoradiography of ^{3}H-QNB and ^{3}H-pirenzepine binding sites. In the present study, an autoradiographic investigation was done using a novel antimuscarinic drug, AF DX 116, as labelled ligand. Receptor binding assay revealed that ^{3}H-AF DX 116 had a single binding site of which Kd and Bmax values were 41.1 nM and 309.2 fmol/mg protein. Brain sections of the rat of an each developmental stage were prepared and incubated with 25 nM ^{3}H-AF DX 116. In the adult rat brain, ^{3}H-AF DX 116 binding sites were richly observed in the superior and inferior colliculi, interpeduncular nucleus, nuclei of the facial and hypoglossal nerves, and moderately in the cerebral cortex (superficial and deeper layers), hippocampus, pontine nucleus and cerebellar cortex. This distribution of ^{3}H-AF DX 116 binding sites was almost consistent with the pattern being expected from those of ^{3}H-QNB and ^{3}H-pirenzepine. From ontogenetic studies, ^{3}H-AF DX 116 binding sites first appeared at 14 days of postnatal days and clearly observed at 21 days of postnatal days. After this stage, they were increased up to 30 days of postnatal days reaching the adult level.

314.18

THREE DIMENSIONAL RECONSTRUCTION OF CHOLINERGIC CELL POPULATIONS IN THE RAT BRAIN: AN INITIAL TEST OF EFFECTS OF A CHRONIC CHOLINERGIC LIGAND K.H. Gylys*, A.P. Painchaud*, S.A. Azizi, D.J. Woodward, L.B. Hersh. (Spon: D.R. Sparkman) *Dept of Cell Biology and Anatomy, Dept of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX.*

The present study was conducted to develop a comprehensive three dimensional computer based representation of the normal cholinergic cell distribution for the entire rat brain using a goat polyclonal antiserum directed against choline acetyltransferase. A second aim was to conduct an initial test of effects of chronic nicotine administration on ChAT-containing structures. Long Evans rats received subcutaneous injections twice daily of either 1.0 mg/kg nicotine or saline for 10-14 days. Results confirm previous reports of cholinergic cell bodies in the: basal forebrain nuclei (medial septal nucleus, diagonal band of Broca, substantia innominata), corpus striatum and nucleus accumbens. More caudally, ChAT-positive cells in the lateral globus pallidus and substantia innominata merge with cells in the basal nucleus of Meynert. The pedunculopontine nucleus is visible in the midbrain. Pontine and medullary sections also display ChAT immunoreactivity in all cranial motor and papasympathetic nuclei. Chronic nicotine administration had no effect on the number of cholinergic somata ($p<.05$) nor in fiber or staining density in comparable brain regions. *Supported by the R.J. Reynolds Tobacco Co., NIDA 2938, and the Biol. Humanics Foundation.*

314.19

NEUROANATOMICALLY SELECTIVE DOWN-REGULATION OF BETA-ADRENERGIC RECEPTORS BY CHRONIC IMIPRAMINE TREATMENT: RELATIONSHIPS TO ^{3}H-IMIPRAMINE BINDING. **G.E. Duncan, I.A. Paul, J.B. Fassberg, K. Powell, W.E. Stumpf, G.R. Breese. (SPON: P.P. Rowell) Biol. Sci. Res. Ctr., School of Med., Univ. of NC at Chapel Hill, NC 27599-7250.**

The down-regulation of beta-adrenergic receptors by chronic imipramine (IMP) treatment was investigated with high resolution autoradiography of ^{125}I-pindolol binding to brain sections. Neuroanatomically selective down-regulation of ^{125}I-pindolol binding was found after chronic IMP treatment. Subdivisions of the amygdala and hippocampus were differentially affected. Significant reductions in ^{125}I-pindolol in the IMP-treated rats were found in the CA-1 stratum radiatum and dentate molecular layer, but not in the CA-3 stratum radiatum of the hippocampus. In the amygdala, the basolateral nucleus exhibited reduced ^{125}I-pindolol binding after IMP treatment but the central and medial nuclei were not affected. Chronic imipramine treatment was also associated with reduced ^{125}I-pindolol binding in layer 1 of the cingulate cortex and layer 3 of the piriform cortex but not in the ventrolateral thalamic nucleus, caudate-putamen, lateral hypothalamus, or layers 2 and 3 of the somatosensory cortex. The regionally selective down-regulation of beta adrenergic receptors by chronic IMP are compared to regional distribution of binding sites for ^{3}H-DMI and ^{3}H-IMP (MH39144 & MH33127.

314.20

THE DIFFERENTIAL BINDING OF AMITRIPTYLINE TO RAT BRAIN MUSCARINIC RECEPTORS. P.S. Goldman* and W.S. Messer, Jr. (SPON: M.E. Velasco). Dept. of Biology and Dept. of Medicinal and Biological Chemistry, College of Pharmacy, University of Toledo, 2801 W. Bancroft, Toledo, OH 43606.

The tricyclic antidepressant amitriptyline is known to inhibit the reuptake of the monoamines serotonin and norepinephrine. Amitriptyline has also been found to bind to muscarinic receptors, which may be responsible for the side effects of the drug, and possibly contribute to its therapeutic activity. The affinity of amitriptyline for muscarinic receptors in rat brain slices was studied using autoradiographic techniques including image analysis. As shown by competitive inhibition of [^{3}H]-1-quinuclidinyl benzilate binding, amitriptyline was found to be more potent than either the M_1-selective antagonist pirenzepine or the M_2-selective antagonist AF-DX 116 based on IC_{50} values.

Muscarinic receptors in the external layers of the cortex bound amitriptyline with the highest affinity (IC_{50} = 65.8 ± 2.1 nM), while the hippocampal regions had somewhat lower affinities (IC_{50} = 96.3 ± 3.4 nM). Amitriptyline bound with lowest affinity in the thalamus and various midbrain regions, such as the paraventricular nucleus of the thalamus and the superior colliculus, which had IC_{50} values of 112 ± 6.8 and 117 ± 32.6 nM respectively. Other midbrain regions displayed higher affinities (e.g., the substantia nigra had an IC_{50} value of 62.8 ± 0.9 nM). The differential binding affinities of amitriptyline suggest that it has higher affinity than either pirenzepine or AF-DX 116 for muscarinic receptors in rat brain with a unique selectivity for subtypes.

Supported by NS 23929

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS IV

315.1

GAMMA-AMINOBUTYRIC ACID OR MU-OPIOID RECEPTOR AUTORADIOGRAPHY IN PALLIDUM AFTER LESIONS IN LATERAL ACCUMBENS AND VENTRAL STRIATUM. L. Churchill, R.P. Dilts and P.W. Kalivas, VCAPP, Wash. State Univ., Pullman, WA 99164.

Do the ventral and dorsal striatopallidal projections parallel each other? Met-enkephalin and γ-Aminobutyric acid (GABA) coexist in both of these pathways. Do the receptors (mu-opioid and GABA, respectively) in the pallidum respond in a similar fashion when the striatal projection is lesioned? Quinolinic acid or nicotinic acid (control) was pressure injected into lateral nucleus accumbens and two weeks later, the rat brains were processed for receptor autoradiography. [3H]quinuclidinylbenzilate (QNB) binding verified the extent of the lesion. [3H]Muscimol binding to GABA receptors and [125I]-Tyr-D-Ala-Gly-MePhe-Gly(ol)(DAGO) binding to mu-opioid receptors were analyzed in ventral pallidum and globus pallidus. [3H]QNB binding decreased in lateral nucleus accumbens and ventrolateral striatum. No differences were observed in [125I]DAGO binding in ventral pallidum or globus pallidus after lesions in lateral nucleus accumbens. [3H]Mus binding decreased in the ventral pallidum after lateral nucleus accumbens lesions but did not change in the lateral globus pallidus when the lesions extended into lateral striatum. These data suggest that the adaptability of GABA receptors to a loss of afferents differs between dorsal and ventral pallidum and that the mu-opioid receptors are not as responsive to loss of afferents as the GABA receptors.

315.2

QUANTITATIVE IMMUNOCYTOCHEMISTRY USING AN IMAGE ANALYZER I. IMAGE PROCESSING AND MEASUREMENT TECHNIQUES. R.R. Mize[1], L.B. Nabors[1], C.J. Jeon,[1] and R.N. Holdefer[2] (SPON: T. Bertorini). [1]Dept. of Anatomy and Neurobiology, Univ. of Tennessee Health Science Center, Memphis, TN 38163 and [2]Dept. of Physiological Optics, School of Optometry, Univ. of Alabama, Birmingham, AL 35294

We have applied image processing procedures to extract and measure immunocytochemically labeled cells and fibers using an image analyzer. Labeled tissue was imaged using a Magiscan IIA image analyzer. Immunocytochemically labeled profiles were enhanced using an edge sharpening operator and extracted using segmentation (thresholding), a procedure which isolates the labeled profiles and removes background. An erosion operator was used to separate cells or fibers that were appossed to one another. A thinning operator was used to reduce fiber width to single pixels in order to measure the length of the labeled fibers. Interactive binary editing was used to remove artifacts. Using this technique, we have measured immunoreactivity in single cells, fibers, and whole fields. The technique was especially useful for estimating the innervation density (fiber area per unit field area) of immunocytochemically labeled fibers. We have found differences in the density of anti-serotonin (5-HT) labeled fibers in different layers of the cat superior colliculus and differences in the size and labeling intensity of anti-GABA labeled neurons in the perigeniculate vs. the lateral geniculate nucleus. The image analysis procedure is a rapid method for measuring the position, geometry, and staining intensity of immunocytochemically labeled tissue.

315.3

QUANTITATIVE IMMUNOCYTOCHEMISTRY USING AN IMAGE ANALYZER. II. ESTIMATION OF GABA CONCENTRATION USING A NON-BIOLOGICAL STANDARD L.B. Nabors*, E. Songu-Mize*, and R.R. Mize, Depts. of Anatomy and Neurobiology and Pharmacology, Univ. of Tennessee Health Science Center, Memphis, TN 38163

We have developed a procedure to estimate the concentration of neurotransmitters in brain based upon the optical density of immunocytochemical labeling measured with an image analyzer. A non-biological standard was used which binds conjugated neurotransmitters. Fifty micron agar sections cut from a block were used as a matrix for the standard. The agar sections were activated with cyanogen bromide/acetonitrile to promote coupling to the antigen. We used gamma aminobutyric acid (GABA) conjugated to bovine serum albumin (BSA) as the antigen. The antibody was directed against this conjugate. Activated agar sections were incubated in serial dilutions of the tritium labeled GABA/BSA conjugate. Some sections were then used to measure the amount of coupled antigen by counting the radioactivity with a liquid scintillation counter. The remaining sections were incubated in the GABA antibody and processed for immunocytochemistry. The optical density of these sections was measured with an image analyzer. A linear relationship was found between optical density and the log concentration of GABA over a range of at least 0.01 to 1 nmol/mg of agar. These results suggest that the concentration of GABA within individual cells and processes can be estimated by comparing the optical density of their label with that of the non-biological standards. Supported by EY-02973 and RR-02800.

315.4

VISUALIZATION OF PROBABLE GABA-B RECEPTOR SITES BY MONOCLONAL ANTI-BACLOFEN ANTIBODIES. G. Holstein, G. Martinelli*, I. Zamir* and P. Pasik, Depts. Neurol., Anat. & Surg., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

The goal of this work was the immunocytochemical visualization of baclofen as a potential marker for GABA-B receptor sites. A series of monoclonal antibodies were raised in mice by standard hybridoma techniques against the L-enantiomer of gamma-amino-beta-(p-chlorophenyl)butyric acid (baclofen) conjugated to keyhole limpet hemocyanin (KLH) using glutaraldehyde. Nine culture supernatants from twice cloned hybrids were selected for their strong reactivity against the glutaraldehyde conjugate of baclofen to human serum albumin (HSA). None of the supernatants tested by enzyme-linked immunosorbent assays (ELISA) showed any reactivity against the glutaraldehyde conjugates of GABA, histamine or monoethanolamine to HSA, suggesting that the antibodies were specific for the baclofen moiety. To evaluate the usefulness of these antibodies, studies of the immunocytochemical localization of baclofen were conducted in rats. Experimental rats were sacrificed 90 minutes after an i.m. injection of baclofen-HCl (1 mg/kg). Both injected and uninjected control rats were sacrificed under ether anesthesia by cardiac perfusion with 2% formaldehyde/0.5% glutaraldehyde in phosphate buffer. Fifty µm sections were exposed to the monoclonal antibodies for 16 hr. The sections were further treated with a rabbit anti-mouse IgG absorbed with normal rat serum, followed by the PAP procedure. Preliminary light microscopic observations suggest the presence of fine immunoreactive elements in discrete brain regions of the baclofen-injected, but not the uninjected rats. For example, cerebellar cortex shows a granular immunostain only in sections from the baclofen-injected animals, particularly prevalent in the granule cell layer. Identically processed sections from the uninjected animals showed no immunoreactivity. Aided by NIH Grants # NS-24656, NS-22953 and NS-11631. L-baclofen was generously provided by Ciba-Geigy, Ltd.

315.5

ANALYSIS OF THE DISTRIBUTION AND DENSITY OF THE GABA/BENZODIAZEPINE/CONVULSANT COMPLEX IN THE RAT CNS. R.W. Olsen[1], R.T. McCabe[2], J.P. Yezuita*[2], and J.K. Wamsley[2]. [1]Dept. of Pharmacology and the BRI, UCLA Sch of Med, Los Angeles, CA 90024 and [2]Dept. of Psychiatry, U of UT Sch of Med, SLC, UT 84132.

The GABA receptor complex which contains pharmacologically recognizable sites that interact allosterically has been the subject of considerable interest. This complex includes GABA, benzodiazepine (BZ), barbiturate, and convulsant sites that are heterogeneously distributed throughout the brain. The binding of ligands which combine with portions of this system was investigated using autoradiographic and homogenate binding techniques. Low to intermediate densities of high-affinity $GABA_A$ receptors in the CNS were labeled with [^{3}H]muscimol. High densities of [^{3}H]bicuculline methiodide and [^{3}H]SR 95531 binding sites (low-affinity $GABA_A$) were found in many regions analyzed. These sites defined by [^{3}H]flunitrazepam binding are coupled to BZ receptors. BZ_1 sites were examined using [^{3}H]2-oxo-quazepam. Convulsant sites which were identified with [^{3}H]TBOB or [^{35}S]TBPS showed intermediate to high levels of binding density in those areas studied. Modulation of [^{35}S]TBPS was examined by including several BZ and barbiturate agents and varied with brain region. Our studies provide a regional comparison of the distribution and density of individual subcomponents of the GABA complex and information regarding relationships of receptor subtypes and GABAergic function.

315.6

DISTRIBUTION OF $GABA_A$/BENZODIAZEPINE RECEPTOR AND NAAG-LIKE IMMUNOREACTIVITY IN THE RAT CNS. P. M. Sweetnam, K. E. Miller, P. A. Gallombardo, J. F. Tallman, J. H. Neale and M. F. Humphrey. Dept. of Neurol. Surg. University of Miami School of Medicine, Miami, FL. 33136; Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508; Dept. of Biology, Georgetown University, Washington, DC 20057.

The modified amino acid GABA and a dipeptide, N-acetylaspartylglutamate (NAAG), are present in relatively high concentrations in the rat CNS. While the inhibitory actions of GABA are well documented, NAAG's role as an excitatory neurotransmitter has yet to be firmly established. However, NAAG's discreet neuronal distribution, vesicular localization and depolarization-mediated release make it an intriguing molecule to study. Using monoclonal antibody Eg, prepared against the purified $GABA_A$/benzodiazepine receptor (Sweetnam, P., et al., Mol. Brain Res., 2:222, 1987), and NAAG antiserum (Cangro, C., et al., J. Neurochem., 49:1579, 1986) we have studied the distribution of both in various regions of the CNS. Our results show that in several regions, e.g., cerebellum, a number of neuronal cell types express both $GABA_A$ receptor and NAAG-like immunoreactivity. However, in other regions, e.g., the spinal cord, the distribution of each is markedly different. These data suggest that in several regions of the rat CNS GABA and NAAG may function in the same local circuitry. Support: The Miami Project to Cure Paralysis; The Daniel Heumann Spinal Cord Research Foundation; DA 02297 to JHN; MH 38813 to JFT.

315.7

IMMUNOHISTOCHEMICAL LOCALIZATION OF GLUTAMIC ACID DECARBOXYLASE (GAD) IN THE INTERPEDUNCULAR NUCLEUS OF THE RAT: AN ELECTRON MICROSCOPE INVESTIGATION. B.A. FLUMERFELT, M.D. KAWAJA* AND A.W. HRYCYSHYN*. Dept. of Anatomy, The University of Western Ontario, London, Canada N6A 5C1

The rat interpeduncular nucleus (IPN), a component of the midbrain limbic system, is composed of an abundant population of topographically organized GAD-positive neurons and a dense plexus of GAD-stained axons. Examination of the ultrastructural localization of GAD-staining in the IPN revealed a variety of arrangements of immunoreactive axodendritic and axosomatic synapses. The lateral, central, intermediate, and rostral subnuclei exhibited numerous GAD-positive somata and dendritic processes. Unstained terminals formed numerous asymmetrical contacts with immunoreactive dendrites and somata, while GAD-stained terminals formed symmetrical axodendritic and axosomatic contacts. GAD-positive terminals were observed throughout the entire centre forming symmetrical contacts with non-immunoreactive dendrites and somata. Immunoreactive myelinated axons were present in the IPN, especially in the lateral subnucleus. This study suggests that a prominent population of GAD-positive neurons contribute to a complex intrinsic GABAergic circuit. These IPN neurons are in receipt of numerous inputs and may give rise to a small GAD-positive projection. (Supported by the Medical Research Council of Canada)

315.8

TWO CLASSES OF CORTICAL GABA NEURONS DEFINED BY DIFFERENTIAL CALCIUM BINDING PROTEIN AND PARVALBUMIN IMMUNOREACTIVITY. E.G. Jones, S.H.C. Hendry and P.C. Emson, Dept. of Anatomy and Neurobiology, University of California, Irvine, 92717 and Institute of Animal Physiology, Cambridge, U.K.

Immunoreactivity for 28kd vitamin D dependent calcium binding protein (CaBP) and parvalbumin (PV) is found in separate sub-populations of GABA immunoreactive neurons in all areas of the neocortex of M. fascicularis monkeys. Most CaBP-positive and PV-positive cells also display GABA immunoreactivity but virtually no cells display both CaBP and PV immunoreactivity. A relatively small number of GABA cells lack immunoreactivity for either peptide. CaBP positive neurons predominate in layers II and IIIA while PV positive neurons predominate in layers IIIB and IV. Both types are found in smaller numbers in deeper layers. Many CaBP positive cells are bitufted and contribute long axon-like processes ascending or descending in the radial fasciculi of the cortex. They are, thus, likely to be double bouquet cells that form GABAergic interlaminar connections. Many PV positive cells have large somata, are multipolar and their axons form pericullular terminals on pyramidal cell somata. They are, thus, likely to be GABAergic basket cells. Supported by NIH Grant NS 21377

315.9

CALCIUM-BINDING-PROTEINS IN THE RAT BRAIN: A MAPPING STUDY M.R. CELIO, Institute of Anatomy University of Kiel, Olshausenstr. 40, D-2300 Kiel

Calcium-binding-proteins (CaBP) translate the information carried by Ca2+ in a meaningful message for the neuron. We are interested in understanding why certain CaBP's occur only in subpopulations of neurons and try to deduce the function of the proteins by studying their neuroanatomical localization. This poster shows that antibodies against the two CaBP's parvalbumin (PV) and calbindin D-28k (28k) are superb new neuroanatomical markers which not only visualize known cells and pathways but even reveal as yet undescribed brain nuclei. PV and 28k occur mostly in complementary systems: the first is abundant in interneurons and the second in projection neurons. PV is preferentially found in a subclass of rapidly firing GABA-positive interneurons; 28k is associated with neurons susceptible to neurodegeneration. The relationship between the distribution of neurons containing these two CaBP's and that of receptors for calcium-regulating hormones and Ca2+-channels is complex. We assume that neurons containing these additional CaBP's are privileged for certain Ca2+-dependent processes and suggest a role of parvalbumin in the control of neuronal excitability.

315.10

NEUROTRANSMITTER PHENOTYPIC EXPRESSION BY EMBRYONIC RAT NEURONS IN DISSOCIATED CULTURE. H. M. Geller, J. P. Grierson, and H. D. Baker#. Dept. of Pharmacology, UMDNJ - Robert Wood Johnson Med. Sch., Piscataway, NJ 08854. #Dept. of Neurology, Burke Rehab. Ctr., Cornell Univ., White Plains, NY 10605

The hypothalamus is a morphologically and functionally diverse region of the brain. This laboratory utilizes dissociated cultures of embryonic rat hypothalamus to investigate the expression of phenotypic properties of hypothalamic neurons in a controlled environment. In this study, we used antibodies directed against tyrosine hydroxylase (TH), GABA and olfactory marker protein (OMP) to investigate the time course and morphological characteristics of expression of these substances. TH^+ neurons appear to form two populations: a large population of small TH^+ neurons which is only observed in young cultures, and a small population of large TH^+ neurons with extensive neuritic ramifications which are first observed at 8 days in vitro and can be identified in older cultures. The overwhelming majority of the neurons in these cultures appear to synthesize immunoreactive GABA; the morphology of these cells is quite heterogeneous. The fact that virtually all neurons are $GABA^+$ would suggest that they contain other transmitters as well. Clusters of small neurons expressing OMP can be identified in cultures as early as 5 days in vitro. One interesting observation is that neurons which express TH and OMP are often found in group rather than as isolated cells, which may suggest a clonal origin for these cells. Supported by NIH NS-25168 and a grant from the Foundation of UMDNJ.

315.11

NEUROPEPTIDE-Y PROJECTIONS FROM LOCUS COERULEUS TO CEREBRAL CORTEX IN THE RAT. B.J. Wilcox and J.R. Unnerstall. Department of Neurology, Case Western Reserve University Sch/Med, Cleveland, OH 44106.

The locus coeruleus (LC) is the sole source of norepinephrine (NE) innervation to the cerebral cortex (CC). In addition to NE, many neurons in this nucleus also contain neuropeptide-Y immunoreactivity (NPY-IR). Thus, the LC may be one source of cortical NPY. We have mapped the rostro-caudal distribution of NPY neurons within the LC by immunofluorescence. In addition, using double-labelling techniques, we have identified NPY containing LC neurons which also project to the cortex. Brains of male Sprague-Dawley rats treated first with Fluorogold, then with colchicine were fixed by perfusion. Frozen sections of LC 10 μm thick were stained for NPY-IR by indirect immunofluorescence. LC neurons labelled after cortical Fluorogold injection were found throughout the dorsal compact LC. NPY-IR neurons were distributed through the entire rostro-caudal extent of the LC including subcoeruleus. Double labelling revealed a small number of neurons containing both NPY-IR and retrograde tracer. Preliminary results show that 10% of Fluorogold labelled neurons in the rostral and caudal poles of the LC also exhibited NPY-IR. In the compact LC, a larger proportion of the Fluorogold labelled cells (up to 30%) exhibited NPY-IR. Thus, LC afferents may contribute to NPY-mediated neurotransmission in the cortex.

315.12

INCREASES IN HIPPOCAMPAL NPY BINDING SITES FOLLOWING LESIONS OF THE LOCUS COERULEUS. J.R. Unnerstall. Departments of Neurology and Pharmacology, Case Western Reserve University Sch/Med, Cleveland, OH 44106.

Neuropeptide-Y (NPY) is found in cortical and hippocampal neurons and is co-localized with epinephrine and/or norepinephrine (NE) in medullary and pontine nuclei. This experiment was designed to determine whether locus coeruleus (LC) NE projections to the forebrain may also utilize NPY. Following 6-OHDA lesions of the LC (2 weeks), rat brains were processed for receptor autoradiography of NPY binding sites using [^{125}I]-PYY (120 pM). Control animals received vehicle injections. Non-specific binding was defined in the presence of 100 nM PYY or 1.0 μM NPY. Given a KD of 60 pM for [^{125}I]-PYY (determined in rat brain sections), these conditions represent an approximate 75% occupancy of high-affinity NPY binding sites. Significant increases in binding ($p < 0.05$) were selectively observed in the CA3 region of the hippocampus (+40%, stratum moleculare; +50%, stratum oriens) in the lesioned animals. Smaller increases (20-25%, $p < 0.10$) were observed in the CA1 region of the hippocampus, subiculum and dorsal medial thalamus. Minimal increases (less than 10%) were observed in the cortex (lamina I), amygdala and dorsal or lateral hypothalamus. These preliminary findings suggest that ascending LC projections to the hippocampus may significantly contribute to NPY-mediated neurotransmission in this structure.

315.13

PNMT, NPY, AND SP-IMMUNOREACTIVE STRUCTURES IN THE MEDULLA OBLONGATA OF THE TREE SHREW. G. Flügge*, S. Behrens*, A. Mittendorf*, F. Pollano * and E. Fuchs. German Primate Center, Göttingen, FRG.

Phenylethanolamine-N-methyltransferase (PNMT), neuropeptide Y (NPY), and substance P (SP) immunoreactive structures were detected in the medulla oblongata (MO) of the tree shrew, a species which provides a model of a small primate brain. Two groups of PNMT immunopositive cells (IC) corresponding to group C1 and C2 of the rat, but no group C3 were found. Most of the IC and immunoreactive terminals (IT) were observed in the solitary tract nucleus (NTS) where they form a ring around the subnucleus gelatinosus (Sg).

In the tree shrew MO, we detected very few NPY-IC but dense patterns of IT. The distribution of NPY-IT showed a great coincidence with PNMT-immunoreactive structures (IS), revealing only some NPY-IT in the Sg.

SP neurons were only found in the raphe obscurus and the hypoglossal nucleus, but there are many SP-IT in the spinal trigeminal nucleus, the vagal nucleus, the NTS, and also in the solitary tract. Most of the SP-IT were found in the medial and intermedial subdivision of the NTS, fewer in the ventrolateral and very few in the Sg. - In summary, the coincidence between the distribution of PNMT- and NPY-IR is in accord with data obtained from the rat whereas the patterns of the IS are similar to those of the rhesus monkey.

315.14

INTRACEREBROVENTRICULAR 6-HYDROXYDOPAMINE AND 5,7-DIHYDROXYTRYPTAMINE DO NOT ALTER ANGIOTENSIN II BINDING SITES IN THE RAT BRAIN. D.E. Walters and R.C. Speth. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520

To determine if the pharmacological actions of centrally administered angiotensin II (Ang II) are mediated via specific Ang II binding sites located on presynaptic monoaminergic nerve terminals, adult male Sprague-Dawley rats (~ 300 gm body wt.) received intracerebroventricular (icv) injections of 6-hydroxydopamine (6-OHDA) or 5,7 dihydroxytryptamine. Subsequently, the pressor and dipsogenic responses to icv Ang II and the specific binding of ^{125}I-sarcosine^1, isoleucine^8 angiotensin II in the hypothalamus-thalamus-septum-midbrain (HTSM) and brainstem were determined. The respective treatments caused reductions in Norepinephrine and 5-hydroxytryptamine levels in the HTSM (62% and 44%) and brainstem (57% and 26%), but did not significantly alter the specific binding of the radioligand in either tissue. Catecholamine depletion in 6-OHDA-treated rats resulted in a 55% decrease in the pressor and drinking response to icv Ang II, although the latter was not significantly different from control. These results indicate that while the central actions of Ang II require catecholaminergic neuron integrity, Ang II does not act directly on catecholaminergic nerve terminals. (Support: NS21305, NS24388 and American Heart Assoc., WA affiliate)

315.15

AUTORADIOGRAPHIC EVIDENCE OF PORCINE BRAIN NATRIURETIC PEPTIDE BINDING SITES IN THE PORCINE AND RAT BRAIN, ADRENAL GLAND AND KIDNEY. K.Shigematsu[2]*, M.Kurihara[3]*, M.Niwa[1], K.Nakao[4]*, Y.Kataoka[1]*, T.Maeda[1]*, H.Tsuchiyama[2]*, M.Ozaki[1]*, H.Imura[4]* and H.Matsuo[5]*, Depts. of [1]Pharmacology 2, [2]Pathology 2, [3]Neurosurgery, Nagasaki Univ.Sch.Med., Nagasaki 852, [4]Second Division, Dept. of Medicine, Kyoto Univ.Sch.Med., Kyoto 606 and [5]Dept. of Biochemistry, Miyazaki Med.College, Miyazaki 889-16,Japan.

Binding sites of porcine brain natriuretic peptide (pBNP) isolated from the porcine brain by H.Matsuo and colleagues (Nature 332,78,1988) were investigated. The tissue sections were incubated with ^{125}I-pBNP and analyzed using quantitative receptor autoradiography. A comparison was made with α-atrial natriuretic peptide (ANP) binding sites. Specific pBNP binding sites were found to be localized in the kidney glomerulus and adrenal zona glomerulosa of rat and porcine. Difference in the localization of pBNP binding sites was noted in the kidney, as indicating that pBNP binding sites were far less abundant in the inner medulla and inner stripe of the outer medulla, areas which were densely labeled by ^{125}I-ANP. In the porcine brain, pBNP binding sites were concentrated in the internal plexiform layer of the olfactory bulb, preoptic medial nuclei, subfornical organ, choroid plexus and suprachiasmatic nuclei. Among areas studied, no differences in the localization were observed when compared with that of ^{125}I-ANP binding sites. As evidence on functional interrelationship between ANP and pBNP has been reported, the present finding enhances our knowledge of the physiology of the natriuretic peptides in the brain and peripheral.

315.16

STAINING OF VASOPRESSINERGIC NEURONS IN ADRENALECTOMIZED RATS USING A VASOPRESSIN ANTI-IDIOTYPE ANTIBODY. D. Berlove, D. Piekut, and K. Knigge. Neuroendocrine Unit, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642

A vasopressin anti-idiotype antibody was generated by immunization of a rabbit with primary anti-vasopressin IgG. This antiserum immunostains neurons in the supraoptic and paraventricular nuclei of the hypothalamus in normal and Brattleboro rats. Staining was reduced or eliminated by preincubation or coincubation of the antiserum with synthetic vaspressin or rat neural membrane in a dose-dependent manner. The anti-idiotype also inhibited binding of ^{3}H-vasopressin to the neural membrane protein in a dose-dependent manner. Preliminary characterization of this antiserum indicates that the anti-idiotype recognizes a putative vasopressin receptor associated with vasopressinergic neurons. Following adrenalectomy it has been established that parvocellular CRF-containing neurons in PVN synthesize vasopressin. We examined the presence of putative vasopressin receptors associated with these parvocellular CRF-vasopressin immunoreactive cells. Rats were adrenalectomized, following a survival period of 72 hours to 8 weeks, brains were stained with the primary anti-vasopressin and the vasopressin anti-idiotype antibody. The vasopressin antisera immunostained neurons in the parvocellular and magnocellular areas of PVN, whereas the anti-idiotypic antibody immunostained magnocellular neurons only. We therefore conclude that the putative vasopressin receptor seen by our anti-idiotype in magnocellular neurons is not present in the parvocellular vasopressin producing neurons of the adrenalectomized rat.

315.17

IMMUNOHISTOCHEMICAL STUDIES ON THE VASOACTIVE SUBSTANCES IN THE HYPOTHALAMIC MAGNOCELLULAR NUCLEI.
H. Yamada*, M. Kawata and Y. Sano*. Dept. of Anatomy, Kyoto Pref. Univ. of Med., Kyoto 602, Japan.

The distribution of such vasoactive substances as serotonin, vasopressin, endogenous digitalis-like substance (EDLS), and atrial natriuretic polypeptide (ANP) in the hypothalamus was studied. The rats and monkeys were transcardially perfused with buffered aldehydes fixatives after they were anesthetized with sodium pentobarbital. Free-floating sections obtained with a cryostat were incubated in the serotonin-, vasopressin-, digoxin- and α-ANP-antibodies, and then subjected immunohistochemically with ABC method. A few serotonin nerve fibers were seen in the paraventricular nucleus, supraoptic nucleus and its accessory nuclei. In these nuclei, EDLS-containing nerve cells were distributed. Moreover, EDLS was co-localized with vasopressin. However, ANP was observed only in the parvocellular component of the paraventricular nucleus. In the rats fed with high sodium purina chow (4 weeks), the immunoreactivities of vasopressin and ANP were increased while those of digoxin (that is, EDLS content) were decreased; however, serotonin-immunoreactivity was not changed.

315.18

DISTRIBUTION OF MONOAMINE AND NEUROPEPTIDE IMMUNOREACTIVE CELLS IN THE CAT PARABRACHIAL PONTINE AREA. K. Elisevich and J. Ciriello. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

The parabrachial pontine area (PPA) surrounding the brachium conjunctivum of the dorsolateral pons has been implicated in pain and cardiovascular control mechanisms. In this study the distribution of perikaraya containing the catecholamine biosynthetic enzymes tyrosine hydroxylase (TH), dopamine-beta-hydroxylase (DBH) and phenylethanolamine N-methyl-transferase (PNMT), in addition to 5-hydroxytryptamine (5-HT), and the peptides substance P (SP) and leucine enkephalin (ENK) in the PPA of the cat were studied immunohistochemically after intracisternal administration of colchicine. Fewer DBH neurons were found in nucleus subcoeruleus (SC), Kolliker-Fuse (KF) and the lateral parabrachial nucleus (PBL) than TH neurons although the nucleus locus coeruleus (LC) contained comparable numbers of both. SP neurons were found in KF with fewer neurons identified in SC, PBL and LC. 5-HT and ENK neurons were more apparent in LC. In light of the neuronal connections of the PPA and its involvement in pain and cardiovascular control, these results suggest the neurochemical substrate for the effects upon analgesia and the circulation. (Supported by HSFO).

LEARNING AND MEMORY: ANATOMY II

316.1

DIRECT PROJECTIONS FROM THE LATERAL PONTINE NUCLEUS TO THE ANTERIOR INTERPOSITUS NUCLEUS: A POTENTIAL CS PATHWAY FOR CLASSICAL CONDITIONING. J.E. Steinmetz and D.R. Sengelaub. Program in Neural Science, Department of Psychology, Indiana University, Bloomington, IN 47405.

A number of studies have suggested that the interpositus nucleus of the cerebellum is a potential site for convergence of CS and US information (i.e., is a potential site for plasticity) associated with classical eyelid conditioning. In addition, previous research has demonstrated that lateral regions of the pontine nuclei (LPN) project essential acoustic CS information to the cerebellum during conditioning (e.g., Steinmetz *et al.*, Proc. Nat. Acad. Sci., 84, 3531-3535). The present study used HRP to determine if cells in the LPN project directly to dorsal regions of the anterior interpositus nucleus.

Stereotaxically placed injections of cholera toxin bound HRP (0.3-2 µl, .2%) were made unilaterally into the region of the interpositus nucleus of rabbits. HRP-labeled cells were visualized with TMB processing.

Our results indicate: (1) Injections which include the anterior interpositus nucleus, dentate nucleus, and overlying regions of the cerebellar cortex (including Larsell's HVI) retrogradely labeled cells in the contralateral LPN and dorsolateral pontine nucleus (DLPN); (2) Injections confined to the interpositus nucleus and white matter above the nucleus also labeled cells located in the LPN and the DLPN; (3) Injections confined to the lateral edge of the anterior interpositus and the medial edge of the dentate nucleus labeled cells in the LPN but not the DLPN. All injections labeled cells in the reticular tegmental pontine nucleus and rostromedial regions of the dorsal accessory olive. These data provide evidence for a direct projection from lateral regions of the pontine nuclei to the deep cerebellar nuclei, a pathway that could be used to relay acoustic CS information to potential sites of plasticity in the cerebellum during classical eyelid conditioning.

316.2

PONTINE PROJECTIONS OF COCHLEAR NUCLEI USING ANTEROGRADE HRP OR PHA-L. J.K. Thompson, D.G. Lavond, C. Weiss and R.F. Thompson. Dept Psych/SGM 501, Univ Southern Calif, Univ Park, Los Angeles, CA 90089-1061.

Previous studies using retrograde fluorescent transport of Fluorogold or of Fast Blue suggest a direct projection from DCN and VCN to posterior aspects of lateral pontine nucleus (LPN). In other studies, field potentials and multiple unit activity can be evoked in the LPN by auditory click stimuli. Both direct electrical stimulation of the LPN as a CS and LPN lesions suggest that it projects essential auditory information to the cerebellar locus of neuronal plasticity for classical conditioning.

The present study was concerned with anatomically confirming the pathway from cochlear nuclei to the pontine region by using anterograde tracing techniques. Rabbits were injected with WGA (pressure injection of 0.1-0.2 uL of 1% or 10% iontophoresis using 14 sec square waves for 10-15 mins) or iontophorectically injected with PHA-L (2.5%) into either the dorsal cochlear nucleus (DCN) or the anteroventral cochlear nucleus (VCN). Survivals were 48 hours after HRP and 14 days after PHA-L injections. The material was processed and was later examined with both light and dark field microscopy.

Supported by NSF BNS8106648 and ONR N0001483K0238 to RFT and NINCDS NS2185303 to DGL.

316.3

UNILATERAL INFERIOR OLIVE NMDA LESION LEADS TO UNILATERAL DEFICIT IN ACQUISITION OF NMR CLASSICAL CONDITIONING. M. Mintz, Y. Yun*, D.G. Lavond and R.F. Thompson. Dept. of Psychol., SGM 501, Univ. Southern California, Los Angeles, CA 90089-1061.

A unilateral lesion of the cerebellum permanently abolishes the NMR only to US presented to the eye **ipsilateral**, but not contralateral, to the side of the lesion. This specialization of cerebellar memory sites might be related to the restricted innervation pattern of cerebellar hemispheres as indeed the climbing fibers to each hemisphere convey mainly the US from the ipsilateral eye. To test this hypothesis, we have measured the conditioning of NMR to US presented to either of the eyes after denervating one cerebellar hemisphere of its US input. The anteriomedial aspect of dorsal accessory olive was localized by antidromic stimulation of cerebellar HVI area and then injected with 40-200 nMol of the neurotoxin NM-DL-A to destroy the cells' somata while sparing fibers of passage from the contralateral olive or other fibers as well. Extensive lesions of the IO prevented acquisition of NMR to the eye ipsilateral to the denervated cerebellar hemisphere. Conditioning to the other eye was normal in all rabbits. It appears that specialization of the memory sites in the cerebellum, in respect to the left vs right US presentation, originate from the distinctive lack of divergence of sensory input from one eye to the bilateral cerebellar hemispheres.

316.4

LESION OF THE CEREBELLAR INTERPOSITUS NUCLEUS ABOLISHES BOTH NICTITATING MEMBRANE AND EYELID EMG CONDITIONED RESPONSES. D.G. Lavond, C.G. Logan, J-H. Sohn, W.D.A. Garner and R.F. Thompson. Dept Psych/SGM 501, Univ Southern California, Univ Park, Los Angeles, CA 90089-1061.

Rabbits were classically conditioned for 5 days at 108 trials per day using the repeating sequence of one CS (1 KHz, 85 dB SPL, 352 ms) alone test trial and 8 paired trials of tone CS and coterminating UCS (left corneal airpuff, 2.1 N/cm^2, 100 ms). Dependent variables were nictitating membrane (NM) extension measured with a minitorque potentiometer and eyelid EMG activity measured with a preamplifier and level discriminator.

Following this all rabbits were anesthetized and lesioned in the left interpositus nucleus of the cerebellum (2 mA, 2.5 min, anodal DC current at AP+0.5, ML-5.0 and DV-14.5 mm from lambda) and allowed 7 days of recovery.

Retesting on classical conditioning demonstrated abolition of both conditioned NM extension and eyelid closure. Averaged and individual trials are presented, demonstrating a high correlation between NM, discriminated and raw EMG before and after the lesion.

Supported by NINCDS 1 R23 NS2185301 to DGL and by NSF BNS8106648 to RFT.

316.5

Filopodia are present in synaptic glomerulae in the tactile learning neuropils of the posterior buccal lobes of *Octopus vulgaris*. J. David Robertson, Psyche Lee and J.Z. Young. Department of Anatomy, Duke University School of Medicine, Durham, N.C.

Tactile learning and memory (L&M) can be blocked in *Octopus vulgaris* by surgical ablation of the posterior buccal (PBL) and subfrontal (SFL) lobes. We have recently found evidence that tactile L&M in *Octopus* can be retarded or blocked by injection of cytochalasin B (CB) into the neuropils of the PBL and SFL without affecting previously established tactile memories. We postulated that L&M depends critically on active extension of filopodia like those of nerve growth cones in specific regions by neurites in response to specific activity. CB is known to cause collapse of filopodia in both neurones and glia in tissue cultures, and this provided the theoretical basis for the CB experiments. We assumed that active extension of filopodia by neurites in learning neuropils is important in L&M. To verify this we have studied serial sections of the PBL neuropil by electron microscopy and found numerous filopodia. These occur in repetitive glomerulae delimited by sheets of glial cells each containing groups of 8-10 presynaptic endings 1-2μm in diameter that surround postsynaptic neurites. These are usually flattened in cross section and often measure ~0.2 x 0.5 μm across. These are connected with nerve fibers 2-3 μm in diameter with prominent neurofilaments and microtubules leading away from the glomerulae. The presynaptic endings contain the usual spherical clear vesicles ~40-60 nm in diameter, some of which are larger and pleomorphic, and groups of spherical dense core vesicles 10-15 nm in diameter. Two sets of serial sections have been analyzed in detail by a computer program for 3D analysis called PC3D (Jandel Scientific) in the laboratory of Dr. Kathleen Smith using a PC's Ltd. 286 computer. Reconstructions were plotted on a HP7475A plotter. Selected profiles of pre- and postsynaptic neurites and glia cells were reconstructed through 52 sections 70 nm thick and 48 sections 50 nm thick. Numerous filopodia like those in neural tissue culture were found in both the pre- and postynaptic neurites and also in glial cells.

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316.6

NEURAL SUBSTRATES FOR LONG-TERM HABITUATION OF THE ACOUSTIC STARTLE REFLEX VISUALIZED USING 2-DEOXYGLUCOSE: REGIONAL CHANGES IN AUDITORY, RETICULAR AND CEREBELLAR SYSTEMS. F. Gonzalez-Lima, T. Finkenstädt* and J.-P. Ewert. Dept. of Anat., Coll. of Med., Texas A&M Univ., College Station, TX 77843, and Dept. of Neuroethol., Univ. of Kassel, Kassel, F.R.G.

Autoradiography with ^{14}C-2-deoxyglucose (2DG) was used to examine the functional activity of the rat brain during habituation of the acoustic startle reflex. Group 1 rats were long-term habituated for one week and injected with 2DG on the last session. Group 2 rats were not pretrained and then were injected with 2DG and stimulated for one session of short-term habituation. Group 3 rats served as unstimulated controls. Long-term habituated rats showed a significantly greater metabolic activation of the auditory system (with the exclusion of the thalamocortical pathway), the cerebellum (deep nuclei, vermian lobules 1-7, lateral hemispheres) and major cerebellar input-output structures (vestibular nuclei, inferior olive, spinal cord). The largest increase was in the lateral superior olive. In contrast, the midbrain reticular formation and its ascending thalamocortical system (anterior, medial, reticular and lateral posterior nuclei, and the frontal cortex) showed significant suppressions in 2DG uptake. The changes revealed by 2DG are the first demonstration of brain substrates with localized metabolic alterations dependent on long-term habituation.

316.7

THE BEHAVIORAL EFFECTS OF CORTICAL SYMPATHETIC INGROWTH (CSI). L. E. Harrell and D. S. Parsons*. Dept. of Neurology, VAMC and Univ. of Ala., Birmingham, AL 35294.

Following cholinergic denervation of the neocortex by nucleus basalis of Meynert lesions (NBML) peripheral noradrenergic fibers are observed to grow into neocortical regions. To ascertain the behavioral effects of cortical sympathetic ingrowth the following experiments were performed.

Sixty-six male adult Sprague-Dawley rats were divided into two Groups. G1 animals underwent training on a standard radial-8-arm maze (RAM) task (i.e., all arms baited) while G2 underwent training on a modified version (i.e., 4 arms baited). After training to a specific learning criterion the animals within each major G were further subdivided into one of three subgroups: Control (CON); NBML (ibotenic 5 ug/ul); NBML + superior cervical ganglionectomy (NBML+Gx). Following surgery, animals were retested until they again achieved learning criterion.

Prior to surgery all animals were able to acquire the task, although those assigned to G2 took longer than those in G1 (mean trials ± SEM: 39.0 ± 5.0 vs 10.2 ± 4.0). Following surgery the NBM animals (34.2 ± 12.2 G1; 59 ± 7.5 G2) took significantly longer to recovery than the NBM+Gx (13.8 ± 2.5 G1; 31 ± 7.1 G2) or CON (10.2 + 2.8 G1; 13 ± 1.6 G2) groups.

The results suggest that CSI, in a manner similar to hippocampal sympathetic ingrowth, may adversely affect behavior. The effect of this neuronal reorganization on behavior following NBML should be considered.

316.8

LOCUS COERULEUS INVOLVEMENT IN CONDITIONED BRADYCARDIA. G.C. Harris and R.D. Fitzgerald*. Dept. of Med. Psychol. Oregon Health Sci. Univ., Portland, OR 97201.

The locus coeruleus (LC) is believed to play a role in the expression of fear. Both NE α-2 agonists and μ opioid agonists are known to have similar effects in suppressing the neuronal activity of LC cells while, corticotropin releasing factor (CRF) is known to enhance the firing of LC cells. This study looked at the effects of the α-2 agonists, Clonidine (3 ug) and UK 14,304 (5 ug), a μ opioid agonist, DALA (10 ug), and the CRF antagonist, Alpha-Helical CRF (9-41) (25 ug) on the development of classically conditioned bradycardia in rats when administered into the rostral fourth ventricle in the vicinity of the LC 5 min prior to training. Conditioning consisted of a discrimination paradigm in which one 6-s tone (CS+) was paired with an aversive US, while a second tone (CS-) was presented alone. An immediate antagonist test and a non-drug test 24 hrs after training were given. All groups were then retrained and the effects of the drugs on an established response was assessed. The administration of DALA, UK 14,304, and Clonidine, were found to decrement both the learning and performance of the conditioned bradycardia. The adverse effect of these drugs on the learning of the bradycardia could be due to their inhibitory action at the LC leading to a loss of LC influences in other brain structures important in the conditioning process. Alpha-Helical CRF appeared to slightly enhance the magnitude of the bradycardia.

316.9

IBOTENIC ACID LESIONS IN THE MAGNOCELLULAR MEDIAL GENICULATE NUCLEUS PREVENT THE ACQUISITION OF CLASSICALLY CONDITIONED BRADYCARDIA TO SINGLE TONES IN RABBITS. N. Schneiderman, C.G. Markgraf, P.M. McCabe, D.R. Liskowsky, and R.W. Winters*. Dept. of Psychology, Univ. of Miami, Coral Gables, FL 33124.

Previous work in our laboratory has demonstrated that ibotenic acid lesions in the magnocellular region of the medial geniculate nucleus (mMGN) prevent the acquisition of differential classically conditioned bradycardia to acoustic stimuli in rabbits. Although lesioned animals were not able to discriminate between a tone paired with shock (CS+) and a separate tone that was not paired with shock (CS-), the animals still exhibited significant bradycardia to both stimuli. This finding suggests the possibility that mMGN is involved in the discrimination between two or more tones, but that simple one-tone conditioning is mediated at a lower level in the auditory system. In order to test this hypothesis, the present study examined the role of mMGN in classically conditioned bradycardia to a single tone.

New Zealand albino rabbits received bilateral lesions via injection of ibotenic acid into either mMGN (n=8) or into control lesion sites (n=16). Following recovery and habituation to the tone CS (560 Hz, 90dB), the CS was paired with periorbital shock (0.5 sec, 3mA) for 30 trials. In half of the control lesioned animals the tone and shock were unpaired in a standard pseudoconditioning paradigm (n=8). Control lesioned animals exhibited bradycardiac responses (mean= -11.6 beats/min) that were significantly greater than the pseudoconditioned group responses (mean= -0.7 beats/min). In contrast, mMGN lesioned animals showed no significant heart rate conditioning (mean= +4.3 beats/min). These findings suggest that neurons intrinsic to mMGN are involved in classically conditioned heart rate responses to single tones as well as in differential conditioning to more than one tone. These results are consistent with results from studies in other species that have demonstrated that MGN is critical for the development of cardiovascular and behavioral conditioned responses to aversive stimuli. Supported by NS 24874, HL 07426, and HL 36588.

316.10

LIDOCAINE INJECTIONS IN RED NUCLEUS ABOLISH NEURAL UNIT MODEL AND CONDITIONED RESPONDING IN STANDARD RABBIT EYEBLINK PARADIGM. A.J. Annala, P.F. Chapman and R.F. Thompson. Program in Neural, Informational, and Behavioral Sciences, University of Southern California, Los Angeles, CA 90089-1061.

The development of a neuronal unit "model" in the interpositus nucleus preceeding the conditioned nictitating membrane response in the rabbit is well established. A similar neuronal model develops in the red nucleus.

Under anesthesia, we inserted cannulae just above the anterior interpositus nucleus or the caudal (magnocellular) red nucleus. We also implanted recording electrodes in the red nucleus or anterior interpositus on the side contralateral to the cannula. After five days of recovery, the animals were trained and then overtrained on a standard eyeblink/nictitating membrane conditioning paradigm with tone and air puff. We then injected small amounts of lidocaine (1 ul of 4 % lidocaine) through the cannulae to produce temporary lesions of the interpositus nucleus or the red nucleus.

Injection of lidocaine in the red nucleus reversibly abolished conditioned responding but did not affect the multiple unit model in the interpositus. Injection of lidocaine in the interpositus reversibly abolished conditioned responding and the multiple unit model in the red nucleus. Control injections of saline resulted in no significant changes. The absence of changes in the interpositus neuronal model following reversible lesions of the red nucleus leads us to conclude the red nucleus is not the site of plasticity.

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316.11

TRANSECTION OF THE MIDDLE CEREBELLAR PEDUNCLE ABOLISHES CLASSICALLY CONDITIONED EYELID RESPONSES IN THE RAT. R.W. Skelton, Dept. of Psychology, Univ. of Victoria, Victoria, B.C., CANADA, V8W 2Y2.

The middle cerebellar peduncle (MCP) has been shown to be essential to classical conditioning of eyelid responses in rabbits (Lewis et al., 1987). The present study tested the effects of electrolytic lesions and knife cuts to the MCP on retention of eyelid conditioned responses (CR) in rats.

Male hooded rats were prepared with subcutaneous EMG electrodes for recording CRs and transorbital electrodes for delivery of eyeshock. In addition, lesion electrodes (or guide cannulae) were implanted bilaterally into the MCP (or 1 mm dorsal to it). After recovery from surgery, each rat was trained to a criterion of 80% CRs in a Pavlovian delay paradigm which paired a 380 msec tone CS with a 100 msec eyeshock unconditioned stimulus (2-4 mA, 60 Hz). The MCP was then destroyed bilaterally by passing anodal current through the lesion electrodes or a knife through the guide cannulae.

Transection of the MCP by either means severely disrupted CRs without impairing either startle responses to the tone or unconditioned responses to the eyeshock. In most cases, the cerebellum itself was undamaged. These results confirm and extend previous evidence that the MCP is a critical afferent to the cerebellum for eyelid CRs. (Supported by a grant from NSERC, Canada.)

316.12

KAINIC ACID LESIONS OF THE CEREBELLAR CORTEX ABOLISH THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE RESPONSE OF THE RABBIT. M.J. Hardiman, M. Glickstein and C.H. Yeo. Dept. Anatomy, University College London, London WC1, U.K.

Discrete aspiration lesions of cerebellar cortex abolish the classically conditioned nictitating membrane response (NMR) of the rabbit (Yeo et al, Behav. Brain Res. 13:261,1984). Thompson and his colleagues failed to replicate this finding. They concluded that the cerebellar cortex is not necessary for conditioning (Lavond et al, Exp. Brain Res. 67:569,1987) and that the cerebellar nuclei are the site of plasticity crucial for NMR conditioning (McCormick and Thompson, Science 223:296,1984).

Here we used kainic acid to make fibre-sparing lesions of cerebellar cortex. NMR conditioning, previously established by pairing light and white noise conditioned stimuli (CS) with periorbital electrical stimulation, was abolished following kainic acid lesions of lobule HVI with damage to adjacent parts of lobule HVII and V. There was no reacquisition within 4000 trials over 20 sessions. Unpaired presentation of the CS revealed no long latency conditioned responses.

These fibre-sparing lesions did not exert their effect by depriving the deep nuclei of their climbing fibre collaterals since the inferior olive was intact. We confirm that the cerebellar cortex is essential for NMR conditioning. (SPON: EBBS)

316.13

SPATIAL NAVIGATION: EVIDENCE FOR CEREBELLAR INVOLVEMENT FROM pcd NEUROLOGICAL MUTANTS. K.M. Hamre, C.R. Goodlett and J.R. West. U. of Iowa, Dept. of Anatomy, Iowa City, IA 52242.

The ability to perform spatial navigation efficiently in the Morris water maze task is known to depend on normal hippocampal function. However, we have found that early postnatal alcohol exposure in rats, which had no significant effects on hippocampal cell numbers but caused severe reductions in cerebellar Purkinje cells, resulted in impairments in spatial navigation. To examine more directly whether Purkinje cell loss may produce spatial navigation deficits, we tested Purkinje Cell Degeneration (pcd) neurological mutant mice, their littermate controls (+/?) and C57BL/6J (B6) mice in the Morris maze. Groups of mice were tested beginning at either 30, 45 or 110 days of age. At all three ages the B6 and +/? groups acquired the spatial problem. The pcd mice failed to improve performance on the hidden platform task, but had only a mild impairment on the visible platform task. To determine whether the impaired spatial performance could be due to secondary cell loss in the hippocampus, pyramidal cells were counted at a mid-temporal level in 60-day-old mice in 5 µm sections. In all of the areas counted (CA4, CA3 and CA1), no differences in cell numbers were observed among the 3 groups. These data suggest that the cerebellum may also be essential to the performance of a spatial navigation task. (Supported by NIAAA grant #AA07313 to J.R.W.)

316.14

LESIONS OF THE PARABRACHIAL NUCLEI INTERFERE WITH THE FORMATION OF CONDITIONED FLAVOUR AVERSIONS. G.M. Martin[1], M. Gans[2], and D. van der Kooy[2]. [1]Dept. of Psychology Memorial University, Newfoundland, [2] Dept. of Anatomy, University of Toronto, Toronto M5S 1A8.

Neuroanatomical data suggest that the parabrachial nuclei could serve an important role in the formation of conditioned flavour aversions since lateral portions of the parabrachial nuclei receive visceral projections and medial portions receive gustatory projections. We removed the neuronal cell bodies of the lateral or medial parabrachial nuclei with bilateral injections of 0.2 ul of 4.0% ibotenic acid. Animals were given a 0.1% saccharin solution to drink and were injected i.p. with 15mg/kg of morpine or a vehicle injection immediately after saccharin removal. Animals with lateral lesions did not form a saccharin aversion, while sham and medial lesioned animals did. During the second training session the animals were permitted access to a 2.0% vinegar solution and were injected i.p. with either 50mg/kg of LiCl, 1 mg/kg of methscopolamine or their saline vehicle after vinegar removal. The animals with lateral lesions did not form as large a conditioned flavour aversion as the sham and medial lesioned animals. Control tests showed that animals with lateral lesions could taste the flavours. These data indicate that the lateral portions of the parabrachial nuclei are important to the formation or recall of conditioned flavour aversions.

316.15

VISUAL CONCEPT OF FOOD/NON-FOOD IN PIGEONS -EFFECTS OF ECTOSTRIATAL LESIONS-
S.Watanabe. Department of Psychology, Keio Univ. Mita 2-15-45, Minato-Ku, Tokyo, Japan.

Two groups of pigeons were trained on food vs non-food objects. An experimental chamber was an operant chamber with a rectangular transparent pecking key behind which a conveyor belt was placed. The conveyor belt had 40 small cubicles each contained food or non-food object. One group was taught to peck the key when foods appeared and the other group was taught to peck when non-food objects appeared. Generalization to new objects was tested when the birds learned the task. Then the ectostriatum or neostriatum was damaged and the birds were retrained. Generalization was tested again after the relearning.

Both groups could learn the discrimination. They showed generalization to the objects which had not been presented during the discrimination training.

After the neostriatal lesion the birds maintained their discrimination, whereas the birds with ectostriatal lesion required longer training to relearn the task. The subjects with damaged neostriatum showed clear generalization after surgery but those with damaged ectostriatum showed weak generalization.

These results suggest that the ectostriatum has an important role in this concept discrimination.

316.16

FOLLOWING BRAIN LESIONS, IF EXPRESSED IN d' TERMS, MEMORY DECAYS AT NORMAL RATES.
J.L. Ringo, Department of Physiology, Univ. of Rochester Med. Ctr., Rochester, NY 14642

Data from the literature on the effect of various brain lesions on recognition memory in the monkey was examined. On the basis of percentage scores the published data can be interpreted to signify that the ability to recognize a previously seen object decays faster in macaques with brain lesions than it does in normal animals.

A re-analysis in terms of the d' of Signal Detection Theory or in terms of an arcsine transform of the percentage values shows, on the contrary, that the rate of "decay" from 0-600 sec is essentially the same in normal animals and in those with lesions. Indeed, the effect of the lesions is fully developed at the shortest times tested, and shows no differential loss as a function of delay between initial presentation and test.

An implication of this result is that the lesion effects are in initial recording or retrieval processes and not in retention or any relatively slow processes following initial presentation.

NEUROGLIA: MYELIN FORMING CELLS

317.1

CLONING AND EXPRESSION OF MYELIN ASSOCIATED GLYCOPROTEIN.
P. Johnson*, M. Tropak*, M. Arquint*, W. Abramow-Newerly*, R. Dunn* and J. Roder, Dept. of Medical Genetics, University of Toronto, Mt. Sinai Hospital Research Institute, Toronto, Ontario M5G 1X5

Myelin associated glycoprotein (MAG) is a 100 kD integral membrane protein that is thought to play a role in the neuron-glial cell interactions that precede myelination. We have cloned and sequenced MAG cDNAs from a rat brain λgt-11 library. Based on the complete cDNA sequence, MAG consists of a large extracellular domain (499 residues and a 90 residue intracellular portion with sites that are phosphorylated *in vivo*. An alternative form of MAG, differing only in the cytoplasmic domain and lacking a prominent site for tyrosine phosphorylation, arises from differential splicing. The expression of the two MAG variants is developmentally and spatially regulated. We have achieved high levels of recombinant MAG protein expression in transfected mammalian and insect cells. Recombinant MAG protein extracted from transfected NIH/3T3 cells and incorporated into liposomes was found to bind specifically to neuronal processes (collaboration with M. Schachner and R. Sadoul, Heidelberg, FRG) and we are currently mapping the binding domains by mutagenesis.

The extracellular domain of MAG contains five tandem repeats, approximately 90 residues in length, which share homology (20-30%) with the variable and constant domains of immunoglobulins and the neural cell adhesion molecule (N-CAM).

317.2

ISOLATION AND PARTIAL CHARACTERIZATION OF cDNA FOR HUMAN MYELIN-ASSOCIATED GLYCOPROTEIN (MAG).
J.R. MOLLER*, R.A. LAZZARINI* and R.H. QUARLES*. (SPON: V.L. Friedrich Jr.) NINCDS, NIH, Bethesda, MD 20892

The myelin-associated glycoprotein (MAG) is an adhesion molecule that is a member of the immunoglobulin superfamily and appears to be involved in the interaction of myelin-forming oligodendrocytes and Schwann cells with axons. There is evidence implicating MAG in the pathogenesis of human diseases such as multiple sclerosis and neuropathy associated with IgM gammopathy. Several laboratories have recently characterized cDNAs from rat brain, but there is evidence for biochemical differences in human MAG which may be relevant to human diseases. Therefore, a cDNA library obtained from the basal ganglia region of a one-day old infant was screened with a ^{32}P-labeled, full-length rat cDNA clone. A positive cDNA was identified and inserted into a pBluescript vector. Double stranded sequencing of the 3'-end of the cDNA has identified 83 bases of the non-coding region, a 16 amino acid signal sequence (D0), 122 amino acids of D1, and 45 amino acids of D2. Compared to the rat sequence, there is one amino acid difference in D0, three in D1, and none in the part of D2 so far sequenced. This corresponds to a 98% amino acid sequence homology. The arg-gly-asp cell attachment site in D1 is conserved, and there is an additional potential glycosylation site in D1 of human MAG in comparison to rat MAG.

317.3

PERIPHERAL NERVE PROTEOLIPID PROTEIN: ISOLATION AND CHARACTERIZATION OF A FULL LENGTH cDNA CLONE.
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Proteolipid protein (PLP), a transmembrane protein, is the major structural protein in central nervous system (CNS) myelin. Three messenger RNAs (mRNAs) encoding PLP are found in rat brain. Two of these, 3.2 and 1.6 kb in length, share a common PLP coding sequence and differ only in their sites of polyadenylation. A third transcript of 2.4 kb, produced by alternative splicing, encodes a PLP with an altered coding region (DM-20). PLP expression has recently been demonstrated in the peripheral nervous system (PNS). The PNS PLP is localized in Schwann cells, but is not found in the myelin sheath. Northern blots of rat sciatic nerve RNA probed with a PLP cDNA reveal mRNAs of 3.2 and 1.6 kb, similar in size to those found in the CNS. In order to understand the difference in PLP localization in the PNS or CNS, we have characterized several PLP cDNA clones isolated from a rat sciatic nerve cDNA library. Two overlapping cDNA clones of 1.4 and 2.4 kb encode an approximately full length cDNA of 3.2 kb. DNA sequence analysis demonstrates that a portion of the 5' untranslated region (70 bp), the coding region (834 bp) and a portion of the 3' untranslated region (500 bp) of the PNS PLP mRNA, are identical to the rat brain PLP cDNA sequence. Restriction enzyme analysis of the remainder of the 3' untranslated region is also identical to the predicted from the rat brain PLP cDNA sequence. These date demonstrate that the PNS and CNS PLP mRNAs have the same primary sequence and suggest that the absence of PLP from PNS myelin is due to mechanisms acting at the posttranslational level.

317.4

THE ROLE OF SUGAR RESIDUES IN THE SORTING OF THE MYELIN PROTEIN, Po.
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The decrease in Po protein of myelin after axotomy is accompanied by a change in its glycosylation pattern, from a complex to a high-mannose type glycoprotein. It has been suggested that this change results in the diversion of Po to the lysosome where it is degraded. To investigate this, we expressed Po in Chinese hamster ovary (CHO) cells, whereby changes in glycosylation could be directly correlated with changes in targeting. A plasmid containing two selectable markers and the Po-cDNA under the control of the metallothionein promoter was constructed and used to transfect CHO cells with complete glycosylation enzymes and mutant CHO cells that synthesized only high mannose type glycoproteins. Initial selection was with the neomycin analogue G418. To obtain an abundance of Po protein for analysis, cells which had co-amplified the Po-cDNA were selected for by the methotrexate/dihydrofolate reductase strategy (stepwise increase of methotrexate up to 1 μM). Western blot analysis showed several clones from both cell lines with increased Po protein (2-5 fold). Immunofluorescence studies with both cell types showed a speckled pattern resembling plasma membrane staining indicating that Po glycoprotein does reach the plasma membrane. The two cell lines permit a detailed assessment of the role of carbohydrates in intracellular sorting of Po protein.
(This work was supported by Grants NS 21700 and NS 22849.)

317.5

TURNOVER OF FATTY ACIDS COVALENTLY BOUND TO PROTEOLIPID PROTEIN (PLP). M.B. Lees and O.A. Bizzozero*. E.K. Shriver Center, 200 Trapelo Rd., Waltham MA 02254.

Fatty acid acylation of PLP occurs actively in adult animals, as well as during rapid myelination, consistent with an active metabolism of the acyl groups. To determine the turnover rate of the acyl groups attached to PLP, forty day old rats were injected intracranially with [^{3}H]palmitic acid and after time intervals from 0.5h to 15 days, myelin proteins were analyzed by SDS-PAGE. Incorporation of the label into PLP reached a maximum after 2 h and declined after 48 h, with a half life of approximately 8 days. Even after 15 days, >87% of the radioactivity associated with PLP could be released with NH_2OH, indicating that it was bound in ester linkage. However, analysis of the ester-bound radioactivity revealed a high degree of fatty acid interconversion. After 1 day, 15% of the protein-bound radioactivity was present in fatty acids other than palmitic whereas after 15 days, more than 50% of the original labeled palmitate had been elongated and/or oxidized. When these values were used to correct for interconversion, the half life of palmitate attached to PLP was only 3-4 days. This rate was slightly faster than that of phosphatidylcholine acyl chains (6-8 days) and much faster than that of the protein moiety calculated under the same experimental conditions (>28 days). These results suggest that acylation of PLP is a dynamic process involved mainly in myelin maintainance and function. Supported by NIH grants NS 16945 HD 05515 and HD 04147.

317.6

IN VITRO FATTY ACID ACYLATION OF Po GLYCOPROTEIN. O.A. Bizzozero* and L. Good*. (Spon: T. Fox). E. K. Shriver Center, 200 Trapelo Rd., Waltham MA 02254.

Po glycoprotein, the major PNS myelin protein, contains 1 mol of ester bound fatty acid and acylation has been demonstrated in vivo. To determine when the fatty acid is attached during the posttranslational processing of the protein, we have studied the kinetics of Po acylation in vitro. Sciatic nerve slices from 10-day old rats were incubated with a mixture of [^{3}H]palmitate and [^{14}C]amino acids and myelin proteins were analyzed by SDS-PAGE. After incubation with [^{3}H]palmitate, most of the protein radioactivity was present in Po (28K). The label associated with Po was identified as palmitic acid bound in ester linkage. The kinetics of entry of newly synthesized and palmitoylated Po into myelin were similar, suggesting that the addition of palmitate occurs near the site of synthesis. Moreover, addition of cycloheximide immediately stopped the synthesis and acylation of total nerve Po, suggesting the absence of a large pool of unacylated Po. Inhibition of protein transport with monensin or colchicine also blocked the entry of acylated Po into myelin. The reaction is a net addition of fatty acid. Developmental studies showed that the synthesis and acylation of Po are maximal during rapid myelination. In conclusion, the results indicate that fatty acids are added to Po in the early stages of the posttranslational processing and not in myelin. Supported by NIH grants 05515 and HD 04147.

317.7

PHOSPHORYLATION OF CHARGED ISOMERS OF HUMAN MYELIN BASIC PROTEIN: EFFECT ON SECONDARY STRUCTURE J.J. Ramwani*, R.M. Epand,* T.E. Mijani* and M.A. Moscarello* (SPON. G. Rajakumar)
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Myelin basic protein (MBP) represents the major extrinsic protein of the myelin membrane. It can be fractionated into several charged isomers (components) on the cation exchange column. In the present study effect of phosphorylation on the secondary structure of four of these components was investigated by circular dichroism.
MBP components were phosphorylated with human brain white matter protein kinase C. The extent of phosphorylation varied considerably from 1.8 moles phosphate/mole protein in component 1 (C-1) to 6.1 moles phosphate/mole protein in component 4 (C-4). All non-phosphorylated components exhibited varying amounts of β structure, random coil and turns, but no α helix. Phosphorylation of these components induced changes in secondary structure and caused an increase in β structure to 40-45% for all components. The increase in β structure could not be reversed by removal of 50% of phosphate by acid phosphatase. The other 50% was inaccessible to the enzyme suggesting it occupies a critical site involved in the stabilization of the β structure. Isolation of the critical site is under way.
In conclusion, components of MBP are phosphorylated to a different extent and phosphorylation of a single critical site may be influencing the stabilization of β structure.

317.8

IDENTIFICATION OF THE Ca^{++}/CALMODULIN-DEPENDENT PROTEIN KINASE IN RAT BRAIN MYELIN FRACTION. Y. Huang* (SPON: M. Stanley). Div. of Neuroscience, New York State Psych. Inst., 722 West 168th Street, New York, N.Y. 10032.

The process of protein phosphorylation and dephosphorylation is considered to be an important mechanism utilized by eucaryotic cells for post-translational regulation of protein function. Many of the biological effects of Ca^{++} in central nervous system are mediated by Ca^{++}/Calmodulin (CaM) dependent protein phosphorylation. Phosphorylation process is also found in brain myelin and myelin basic protein (MBP) is a major phosphoprotein. In the course of our characterization of post synaptic densities (PSDs) in human and rat brains, we have detected the presence of a Ca^{++}/CaM-dependent protein kinase capable of phosphorylation of polypeptide doublets of 48/50 kDa and 56/58 kDa in rat brain myelin fraction. Phosphorylation of these polypeptide doublets is stimulated in the presence of the Ca^{++} ions and CaM and is inhibited by the addition of the ganglioside GM1. The phosphorylation assay included 50 mM PIPES (pH 7.0), 0.5 mM Ca^{++}, 0.2 mM EGTA, 1 mM DTT, 1 mM Mg^{++} and 13 uM $^{-32}$P-ATP, and various effectors including CaM and gangliosides (10 ug). Proteins were subjected to gradient PAGE containing SDS. Gels were then exposed to X-ray film for autoradiographic localization of phosphoprotein bands. The ratio of intensity of these phosphoprotein bands between polypeptide doublets of 48/50 kDa and 56/58 kDa is 3 : 1 while 48 kDa and 50 kDa is 1-1.5 : 1 in autoradiography. The preliminary results suggest that this may be a Ca^{++}/CaM-dependent protein kinase with multiple isoenzymatic forms and/or its location in brain myelin fraction may produce differential changes in its activity.

317.9

EXPRESSION OF TRANSFERRIN AND THE TRANSFERRIN RECEPTOR BY OLIGODENDROCYTES IN VITRO. W.P. Bartlett and J.R. Connor. Dept. Anat., M.S. Hershey Med. Ctr., Hershey, PA 17033.

Transferrin, the iron mobilization protein, has been found in oligodendrocytes in the rat, mouse, and human CNS. We have undertaken the following experiment to demonstrate 1) whether oligodendrocytes grown in vitro accumulate transferrin (Tf) and express the Tf receptor, 2) the temporal sequence of Tf and Tf receptor appearance in relation to galactocerebroside and myelin basic protein. Cells were examined immunohistochemically in dissociated mixed glial cell cultures from 3-4 day old mouse pups. Tf receptor positive cells appear before the expression of GalC immunoreactive cells; an oligodendroglial marker. These cells are small, round, process-bearing cells which lie on the surface of the astrocytic bed layer. When immunostained prior to fixation, the pattern of staining is restricted to the soma and occasionally the proximal portion of a process. The Tf receptor positive cells also immunoreact with antisera to MBP and GalC. Furthermore, MBP and GalC cells immunostain with Tf. However, while MBP and GalC positive sheets of myelin-like membrane are visible, Tf is confined mostly to the perikaryal cytoplasm. This study reveals that Tf and its receptor are present in oligodendrocytes in culture and suggests that the appearance of the Tf receptor may be an early indication of a differentiated oligodendrocyte. Supported by NMSS RG1998 (WPB) and NS22671 (JRC).

317.10

TRANSFERRIN AND ITS RECEPTOR ARE EXPRESSED BY MYELINATING SCHWANN CELLS. H.H. Lin*, S. Toland*, and J.R. Connor. Department of Anatomy, M.S. Hershey Medical Center, Hershey, PA 17033.

A substance from ground sciatic nerve called "sciatin" was shown to be capable of replacing the requirement for transferrin (Tf) in cultures of chicken myoblasts. This substance was later shown to be Tf, but the source of the Tf in the sciatic nerve has not been determined. In this study, we demonstrate immunohistochemically that Tf and the Tf receptor are expressed by Schwann cells in the sciatic nerve, but not by those in the thoracic sympathetic trunk or vagus nerve. Following crush of the sciatic nerve Tf, the Tf receptor and galactocerebroside (GalC) are not detectable in the distal nerve segment. By 5 weeks, post-crush some GalC and Tf receptor staining is present, but not Tf. GalC, Tf receptor, and Tf are all present by 8 weeks after the crush injury, but the immunohistochemical staining pattern is abnormal. The abnormal staining pattern continues into the 10th week post-crush. The results of the crush injury study show that the expression of the Tf receptor precedes the accumulation of Tf by Schwann cells and is at least coincident with the expression of GalC. These data support the general hypothesis that Tf accumulation by myelinating cells (oligodendrocytes and and Schwann cells) is a necessary condition for the production of myelin.
Supported by NIH grant NS-22671.

317.11

Monoclonal Antibody OLIGO Recognizes Presumptive Oligodendrocytes Prior to Myelination.

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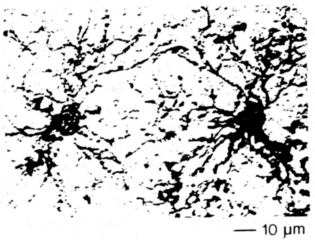
Previous work has shown that the monoclonal antibody OLIGO recognizes mature oligodendrocytes, myelin sheaths and immature myelin related oligodendrocytes in tadpoles.[1] The present immunocytochemical study focuses on cells that have not formed myelin but are recognized by OLIGO. The cells formed networks of immunostained processes (Figure). These processes closely resemble processes elaborated by rat oligodendrocytes in cultures free of neurons.[2] Thus our cells may be premyelinating oligodendrocytes. The longest processes of our OLIGO stained cells had a range of 48 μm +/− 10 μm (SD) (11 cells, 2 tadpoles). Process from adjacent stained cells mingled and appeared to contact each other. The observed overlap of processes suggests that presumptive oligodendrocytes may interact during early stages of development.



Supported by NIH, NMSS & VA.

(1) Steen, Kalghatgi & Constantine-Paton (1987) *Soc. Neurosci. Abstr.* 13:1691. (2) Kachar, Behar & Dubois-Dalcq (1986) *Cell Tissue Res.* 244:27.

317.12

THE EXPRESSION OF MYELIN ANTIGENS, GFAP, AND TRANSFERRIN AND ITS RECEPTOR IN THE DEVELOPING RAT OPTIC NERVE. J.R. Connor and H.H. Lin* (SPON: R. Bohn). Dept. of Anatomy, M.S. Hershey Medical Center, Hershey, PA 17033.

Extrinsic factors (non-neuronal) are thought to exist which influence or possibly control the process of myelination. Recently, the iron transport protein, transferrin (Tf), has been found in oligodendrocytes suggesting that iron and Tf could play a role in myelinogenesis. In the present study, the developing rat optic nerve was examined immunohistochemically with antibodies to myelin basic protein; MBP and the transferrin receptor; Tfr, galactocerebroside, Tf, and glial fibrillary acidic protein. Tfr and Tf positive cells were seen near blood vessels at PND 8. Patches of MBP and GalC positive fibers were also present. The number of Tfr positive cells peaked between PND 15-21 and were generally perivascular. The number of Tf, GalC and MBP cells continued to increase into adulthood and were found throughout the optic nerve. These results demonstrate that Tfr positive oligodendrocytes appear at the time myelin begins and are most numerous during the highest rate of myelination and then decrease thereafter. Furthermore, the presence of Tf in oligodencrocytes precedes the expression of MBP and GalC in oligodendrocytes. These data are further support of the hypothesis that Tf accumulation by oligodendrocytes is a necessary condition for the process of myelination.

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317.13

In Vitro Analysis of Neuroglial Cells Isolated During Demyelination and Remyelination.

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During development a subpopulation of neuroglial cells (O-2A progenitors, oligodendrocytes, and type-2 astrocytes) is involved in CNS myelination. To analyze the potential role of this subpopulation in the course of demyelination and remyelination we induced demyelinating lesions of the spinal cord in 28d old C57Bl/6 mice by intracerebral injection of MHV-A59 coronavirus. At 4-5 wks post-infection (p.i.) vacuoles and demyelinated axons were evident in spinal cord white matter. Remyelination was already beginning and resulted in extensive myelin repair in the following 8-12 wks.

Neuroglial cells were isolated from spinal cord by combined enzymatic and mechanical dissociation along with Percoll gradient centrifugation, cultured for 1d, and stained simultaneously for O4 antigen, galactocerebroside (GC), and glial fibrillary acidic protein (GFAP) with 3-color immunofluorescence.

Control cultures contained mainly process-bearing cells which expressed O4, a developmental marker for intermediate and mature O-2A lineage cells. The majority of O4+ cells also expressed GC, an oligodendrocyte marker. Remaining O4+ cells were either O4+GFAP+ (a phenotype of type-2 astrocytes) or O4+ only (a characteristic of an intermediary in the O-2A pathway). Cultures of lesioned spinal cord (4-5 wks p.i.) exhibited a striking increase in the number of O4+GFAP+ and O4+ only cells. More O4+GC+ cells were apparent also, however this increase was proportionally smaller than was noted for the other two phenotypes. O-2A lineage type cells labeled *in vivo* with 3H-thymidine could be isolated from lesioned but not control adult spinal cord. These findings may be explained by gliogenesis and/or phenotypic changes of O-2A lineage cells which may occur in the course of remyelination, and which can be analyzed in this *in vitro* system.

317.14

EFFECT OF HISTAMINE ON PROLIFERATION OF CULTURED SCHWANN CELLS. Brian M. Gordon* and Jun E. Yoshino. Program of Neuroscience, Dept Psychology, Colgate Univ, Hamilton, NY.

Axolemma-(AEF) and myelin-enriched (MEF) fractions stimulate the division of Schwann cells (Yoshino et al., 1984, J Cell Biol. 99:2309). The addition of ammonium chloride, phorbol esters, calcium chelators, or lithium modulates the mitogenic activities of these fractions. We examined the effect of histamine, a secretory product of mast cells, on Schwann cell proliferation, because mast cells have been identified as having possible roles in the origin of neurofibromas and during demyelinating diseases.

Cultured Schwann cells were prepared from 3d rat sciatic nerve. Schwann cells treated with histamine (0.5-2.0mM) incorporated the same levels of ^{3}H-thymidine as controls (no histamine). The amount of ^{3}H-thymidine incorporated by Schwann cells treated with 2mM histamine and the MEF was greater than the level of ^{3}H-thymidine accumulated by those cells receiving only myelin. The increase in ^{3}H-thymidine uptake ranged from 20%-50% depending upon the concentration of the MEF. By autoradiography, 2mM histamine alone did not increase the number of labelled cells. The addition of 2mM histamine to MEF treated Schwann cells resulted in a 50% increase in labelled cells. In contrast, the addition of histamine did not alter the mitogenicity of the AEF. Thus, histamine specifically enhances Schwann cell proliferation induced by MEF, and may play a role in cellular proliferation occurring in certain disease states.

Supported by Colgate Research Council.

317.15

DEVELOPMENTAL REGULATION OF CHANNEL EXPRESSION IN OLIGODENDROCYTES. H. Sontheimer*, J. Trotter*, M. Schachner and H. Kettenmann* (SPON: G. ten Bruggencate). Dept. of Neurobiology, Univ. Heidelberg, INF 364, 6900 Heidelberg, FRG. We characterized membrane currents in cultured brain derived murine oligodendrocytes at different developmental stages, using the patch-clamp technique. Recordings were made from O10-positive/O4-positive more mature oligodendrocytes with large somata (>15 um) and many processes, O4-positive/O10-negative immature oligodendrocytes with smaller somata (10-15 um) and less than 5 processes, and O4-negative cells which probably are precursor cells with small somata (<10 um) and 1 to 3 processes. In more mature oligodendrocytes we found no voltage activated currents, but a marked inward rectification. Currents were 4-AP insensitive. In contrast, immature oligodendrocytes expressed an inactivating and a noninactivating K^+-current which could be completely blocked by 4-AP and TEA as previously described for astrocytes. The current-voltage curve showed a strong outward rectification. In addition, we observed sodium currents which peaked at about -20 mV in 4 out of 39 immature oligodendrocytes. Precursor cells expressed K^+-currents similar to those seen in immature oligodendrocytes, but in addition sodium currents were always observed. Thus channel properties of oligodendrocytes are developmentally regulated, with sodium and voltage activated potassium currents expressed early and lost during differentiation, and with voltage insensitive currents appearing at later stages.

317.16

MYELIN PROTEIN BIOSYNTHESIS BY CERVICAL SYMPATHETIC SCHWANN CELLS IN VIVO AND IN VITRO. K. R. Brunden*, A. J. Windebank, and J. F. Poduslo. Membrane Biochem. Lab., Depts. Neuro. & Biochem./Molec. Biol., Mayo Fdn., Rochester, MN 55905

More than 99% of Schwann cells (SCs) from the cervical sympathetic trunk (CST) are non-myelinating. Immunoblot analysis of endoneurial homogenates indicates that the levels of the 28 kD P_0 glycoprotein and the 14-21 kD MBPs found in adult rat CST are very low compared to those found in the sciatic nerve. Likewise, [^{3}H]Man and [^{3}H]amino acid incorporation studies reveal that the level of P_0 biosynthesis in the CST is greatly reduced relative to that of the sciatic nerve. SCs derived from adult CST will myelinate dorsal root ganglia (DRG) neurite networks if grown in the presence of serum. Immunoblot analysis of these cultures demonstrates the presence of both P_0 and MBPs. CST SCs will not myelinate DRG neurites if grown in the absence of serum, but these SCs do synthesize a 28 kD glycoprotein at levels comparable to those seen in myelinating cultures when assayed by [^{3}H]Man incorporation. This glycoprotein co-migrates with [^{3}H]Man-labeled P_0 from myelinating cultures during SDS-PAGE, and like P_0 it shows a reduction of M_r in the absence of reducing agent. A similar glycoprotein is synthesized by SCs derived from 15-day embryonic DRG co-cultured with neurites in the absence of serum. The active biosynthesis of a putative P_0 species by the cultures grown without serum would suggest that contact with myelin-supporting axons is sufficient to induce myelin protein biosynthesis even if myelin assembly is not occurring. (NS20551)

317.17

MYELIN DEGRADATION IN SCIATIC NERVE EXPLANTS.
D.L. Novicki*, J.F. Goodrum* and T.W. Bouldin*. (SPON: E. Costantino-Ceccarini). Pathology Dept. and Biological Sciences Research Center, University of North Carolina, Chapel Hill, NC 27599-7525

Studies from several laboratories have variously attributed the phagocytosis and degradation of myelin during Wallerian degeneration solely to fibroblasts, hematogenous cells, vascular pericytes, Schwann cells, endoneurial cells that have transformed into phagocytes, or to combinations of these cell types. This controversy is unlikely to be resolved by studies based solely on morphological evaluation of degenerating nerve in vivo. We have been using explant cultures of sciatic nerve endoneurial tissue from 35-45 day old male Long-Evans rats to investigate the potential of endoneurial cells to engulf and/or degrade myelin in the absence of hematogenous cells.

Peripheral nerve explants were maintained for 1 day to four weeks and examined for myelin P_0 protein by SDS-PAGE. The P_0 levels decreased approximately 50% after 4 weeks in culture. Ultrastructural examination of explants indicated that the cellularity of the explants decreased as time in culture increased and that some myelin remained undegraded. Fibroblasts, Schwann cells and macrophage-like cells all contained myelin debris. Nonspecific esterase activity, which is a macrophage marker, was present in some Schwann cells as well as in macrophage-like cells. Autoradiography following exposure to ^{3}H-thymidine demonstrated that cells within the explant and cells which had grown out were capable of proliferating. Studies are underway to determine if the macrophage-like cells are capable of proliferation in the presence or absence of conditioned medium containing colony stimulating factor.

We conclude that rat endoneurial tissue contains cells that are capable of phagocytosis and breakdown of myelin, and that these processes can occur in nerve in the absence of infiltrating hematogenous elements. Supported by ES 01104.

317.18

THE DEVELOPMENT OF OLIGODENDROGLIA IN CULTURE.
S.E. Poduslo, K. Miller*, and C.H. Pak*. Johns Hopkins Univ. Sch. of Med., Dept. Neurology, Baltimore, MD 21205.

Oligodendroglia elaborate extensive quantities of membranes which enfold segments of axons to form the multilamellar highly compacted myelin sheath. It is possible to obtain oligodendroglia by either differential plating methods or by bulk-isolation techniques. The differential plating method involves primary cultures prepared from neonatal rat brain. The bulk-isolation technique uses brain tissue from actively myelinating adolescent rat or dissected white matter from bovine brain. The rat oligodendroglia elaborate extensive networks of processes in culture but produce no myelin. Bovine oligodendroglia in suspension cultures produce whorls of membrane which biochemically appear to be early forms of myelin. Thus the rat oligodendroglia appear to be less differentiated than bovine cells. The different stages of oligodendroglia were analyzed for the synthesis of specific lipids in the presence of various agents known to affect cell differentiation. In this study, evidence of further development in culture would be increased synthesis of cerebrosides, a myelin component. Retinoic acid and thyroid hormone appeared to have a positive effect on cerebroside synthesis, while 5-azacytidine and phorbol esters had a negative effect. Ketone bodies had a slight stimulatory effect. Other agents such as sodium butyrate had no effect. (Supported by grants from NIH NS 14577 and HD 16956).

317.19

CHANGES IN CNS MYELIN FORMATION IN RESPONSE TO A REDUCTION IN OLIGODENDROCYTE PRECURSORS. T.J. Sims and S.A. Gilmore. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

Exposure of lumbosacral spinal cords of 3-day-old rats to ionizing radiation induces a marked reduction in glia. Although myelin formation is delayed in both dorsal (DF) and ventral (VF) funiculi, the axons remain healthy and increase in diameter. Reconstitution of the glial population occurs with time but differs between these funiculi. Myelin formation by oligodendrocytes and Schwann cells occurs dorsally, whereas oligodendrocytes are responsible for this delayed myelination ventrally. Differences in the pattern of oligodendrocyte myelination are observed more frequently in the DF where areas of white matter myelinated by Schwann cells are interspersed with those sparsely populated by oligodendrocytes. Myelin in these latter areas appears to be derived from two different forms of oligodendrocytes. One appeared normal in that large cytoplasmic processes extended short distances and gave rise to myelin sheaths of normal thickness. Emanating from the second form of oligodendrocyte were many small processes which extended for considerable distances amongst the axons and formed myelin sheaths that were extremely thin relative to axonal diameter. The general impression is that this oligodendrocyte process "overextended" itself in order to myelinate a sizeable number of large axons. The explanation for the two different forms of oligodendrocytes is not clear nor is the dorsal-ventral difference in their frequency. Perhaps the normal appearing one was present and involved in myelin formation prior to irradiation, whereas the second form, generated after exposure, matured in an environment of large diameter, non-myelinated axons expressing an affinity for oligodendrocytes which exceeded that existing at the usual time of myelination. (Supported by NIH Grant - NS 04761).

317.20

GENERATION OF CYTOTOXIC LYMPHOCYTES FOR HUMAN GLIAL CELLS IN CULTURE. T.C.G. Ruijs*, A. Olivier*, M.S. Freedman*, J.P. Antel. Montreal Neurological Institute, McGill Univ., Montreal, Quebec, Canada H3A 2B4.

Cellular immune mechanisms directed against the myelin-forming oligodendrocytes (OGC) could contribute to tissue injury in human demyelinating disease. We attempted to measure susceptibility of OGC to cell-mediated cytotoxicity using OGC enriched adult human glial cell cultures derived from surgically resected tissue in a ^{51}Cr release assay. In a lectin-dependent, non-MHC restricted system, Concanavalin A stimulated lymphocytes were shown to lyse glial cells (mean specific lysis 30% in 4:1 ratio). Allogeneic CD8+ lymphocytes activated in a mixed lymphocyte reaction against glial cell donor's lymphocytes also induced specific cytolysis of the donor's glial cells. The CD8+ subset comprises MHC class I restricted antigen specific cytotoxic T lymphocytes, as well as non-MHC restricted natural killer (NK) cells. OGC were not lysed by non-activated lymphocytes, the usual means to assay for NK cell activity. Immunohistochemical studies indicated that the OGC (GalC+ cells) express MHC class I but not class II antigens in vitro.

EXCITATORY AMINO ACIDS VI

318.1

ION FLUX AND PATCH CLAMP MEASUREMENTS OF PARTIALLY PURIFIED AND RECONSTITUTED GLUTAMATE-ACTIVATED ION CHANNELS. E.K. Michaelis, A.M. Ly*, M. Uto* and T. Kuwana*. Department of Pharmacology and Centers for Biomed. and Bioanalyt. Res., Univ. of Kansas, Lawrence, KS 66046.

Treatment of rat brain synaptic membranes with the non-ionic detergents Triton X-100 or n-octylglucoside in the presence of neutral phospholipids or glycerol plus phospholipids, led to successful solubilization and subsequent reconstitution of glutamate-activated Na^+ channels into liposomes. Partial purification of glutamate-binding proteins through affinity batch chromatography as described previously (Chen et al., 1988, J. Biol. Chem. 263, 417) produced both enrichment of glutamate-sensitive ion channels in reconstituted liposomes and of the ~ 70 and 63 kDa glutamate-binding proteins. Few other proteins were also detected in a number of our preparations (e.g. 90, 47, and 32 kDa). L-Glutamate (8-10 μm) caused a transient transmembrane potential in liposomes that was detected as the uptake of the anion probe [^{14}C]SCN^-. The receptor agonists ibotenate, quisqualate and N-methyl-D-aspartate also enhanced SCN^- uptake whereas the antagonists L-glutamate diethyl ester and D-2-amino-5-phosphonopentanoic acid blocked the effect of L-glutamate on SCN^- flux. L-Glutamate-activated ion channels could be detected in membrane bilayers formed at the tip of patch pipettes from liposomes reconstituted with the partially purified glutamate-binding proteins. [Supported by grants DAAL 03-86-K-0086 and 03-88-K0017 from ARO; AA 04732 from NIAAA].

318.2

ACTIONS OF CNQX IN AREA CA1 OF RAT HIPPOCAMPAL SLICES. G.L. Collingridge*, S.N. Davies*, R. Yates*, J.F. Blake*, and M.W. Brown* (SPON: W.W. Anderson), Departments of Pharmacology and Anatomy, University of Bristol, BS8 1TD.

We have studied the effects of 6-Cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX) in hippocampal slices (in 1 mM Mg).

CNQX (1-30 µM) produced a parallel shift in the log-dose response curves and gave the following Kd values (µM): α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), 2; kainate, 2; N-methyl-D-aspartate (NMDA), >30. Low frequency (0.033 Hz) synaptic responses, elicited by low intensity stimulation of Schaffer collateral-commissural fibres, were blocked by 10 µM CNQX. However, with high intensity or high frequency (100 Hz, 0.5-1 s) stimulation a component remained which was reversibly blocked by a selective NMDA antagonist.

Field EPSPs were evoked by stimulation of two independent sets of convergent fibres. Long-term potentiation was induced specifically in one set and the stimulus intensity adjusted so that both pathways elicited EPSPs of a similar size. CNQX blocked both the potentiated and the non-potentiated response in parallel.

We conclude (i) NMDA receptors can be synaptically activated after blockade of non-NMDA type receptors and (ii) the same type of CNQX-sensitive receptor is likely to mediate both potentiated and non-potentiated responses.

CNQX was generously provided by Dr. T. Honoré (Ferrosan).

318.3

Mg^{++}, EXCITATORY AMINO ACIDS, AND DORSAL ROOT POTENTIALS IN THE FROG SPINAL CORD. J.C. Hackman and R.A. Davidoff. Neurophysiology Lab., VAMC and Dept. of Neurology, Univ. of Miami School of Med., Miami, FL 33101.

The basis for the long duration dorsal root potentials (DRPs) in the in vitro frog spinal cord is unexplained. We investigated the role of Mg^{++} since most frog Ringer's solutions omit Mg^{++} although frog CSF contains 1mM Mg^{++}.

Rana pipiens were anesthetized on ice and the hemisected spinal cord and dorsal roots (DR) placed in a sucrose gap chamber. The peak amplitude, duration, and area of DRPs evoked by single DR stimuli were stored in a computer and analyzed using Asyst software.

DRP duration ($\bar{X}$8.6±2.1 s, HCO_3-buffered medium, 0mM Mg^{++}) and area (30.0±0.5 V/ms) were decreased by 75% and 80% in 1 mM Mg^{++}; the peak amplitude (14.1±0.7 mV) was only reduced by 24%. APV (10uM) diminished DRP amplitude and area by only 14% and 40% which suggests that 1 mM Mg^{++} has effects other than NMDA antagonism. Kynurenate (KYN, 2mM) reduced the DRP amplitude to 31±5% of control values. Further addition of bicuculline (25uM) reduced the DRP to 31±7% of the KYN amplitude suggesting that about 1/3 of the GABA-DRP is independent of excitatory amino acid transmission. 20mM Mg^{++} diminished the DRP to nearly the same level as did the combination of KYN and bicuculline.

In sum, 1 mM Mg^{++} dramatically decreases the duration and area of the amphibian DRP. In addition, synaptic release of excitatory amino acids appear to contribute to nearly 70% of the DRP generation. (Supported by VAMC Funds MRIS #1769 and #3369 and USPHS grant #17577)

318.4

COMPARISON OF CURRENTS PRODUCED BY ELECTROGENIC UPTAKE OF L-GLUTAMATE AND L-ASPARTATE IN GLIAL CELLS ISOLATED FROM TIGER SALAMANDER RETINA. H. Brew* (SPON: G. Hall) Dept. of Physiology, University College London, London, U.K.

L-glutamate evokes an inward current in whole-cell clamped retinal glial (Müller) cells, due to the activation of an electrogenic (and sodium-dependent) glutamate uptake system (Brew & Attwell, 1987). I report here that the Müller cell currents evoked by aspartate, I(asp), differ from those evoked by glutamate, I(glut).

The magnitude of currents evoked by L-aspartate (ASP) or L-glutamate (GLUT) obey Michaelis-Menten kinetics (apparent K_m is 20μM for glutamate, 3μM for aspartate, independent of voltage). The maximum currents evoked by these agonists are strongly voltage-dependent, with I(glut) having a different voltage-dependence than I(asp).

When 30μM GLUT and 30μM ASP are applied to a Müller cell simultaneously, the current produced is similar (in size and voltage-dependence) to that produced by 30μM ASP alone. This suggests that mostly ASP is binding to the carriers available and being transported, resulting in aspartate-type currents (as is predicted if ASP and GLUT are transported by the same uptake system, with K_ms as above, and differences in their cross-membrane transport).

The carrier's different affinities for these two neurotransmitters may mean that if they are ever co-released at synapses, aspartate could inhibit glutamate uptake by glia, thus enhancing glutamate's postsynaptic action. (Supported by the MRC and the Wellcome Trust)

318.5

INTRACELLULAR RECORDINGS OF A SLOW NMDA RECEPTOR MEDIATED SYNAPTIC POTENTIAL IN INFERIOR COLLICULAR SLICES. M.G. Pierson, K.L. Smith, and J.W. Swann. Wadsworth Center, N.Y. State Dept. of Health, Albany, N.Y. 12201

Using in vitro slices from immature rat brain, we have studied local current-stimulated events in disinhibited (2.5 μM bicuculline) inferior colliculus. Using extracellular electrodes (2 M NaCl) we recorded an initial field potential and a very slow (1-10 sec) field potential which was accompanied in some instances by concurrent afterdischarges. Intracellular recordings (4M KAc or 1M CsAc) revealed coincident synaptic events which included: 1) a bicuculline-sensitive ipsp (equilibrium potential, -70mV), 2) a fast voltage-independent epsp (equilibrium potential, 0mV) and 3) a strikingly large (eg. 30 to 40 mV), longlasting (up to 10 sec) synaptic potential. The latter potential was reversibly and selectively abolished by 10 μM micro-ejected droplets of D-APV, 2) exhibited voltage-dependency and 3) had an equilibrium potential of approximately 0mV. Thus we infer it to be an NMDA synaptic response. Because it was also reversibly abolished by increasing extracellular Ca^{++} (from 2 to 6 mM), it is likely that the slow synaptic potential is polysynaptic in origin. The extraordinary duration of the potential is suggestive of reverberating local circuit activity.

318.6

TWO DISTINCT QUISQUALATE RECEPTORS IN ISOLATED RETINAL GANGLION CELLS FROM RAT. Elias Aizenman, Andreas Karschin*, and Stuart A. Lipton. Div. of Neuroscience, Children's Hosp. and Harvard Med. Sch., Boston, MA 02115, and Max-Planck Inst. für Hirnforschung, D-6000 Frankfurt, FRG.

We have examined with patch electrodes the inhibitory actions of kynurenate and FG9065 (CNQX) on responses elicited by quisqualate and AMPA in isolated retinal ganglion cells from rat. In 16 of the cells (type *A* cells) in which both agonists were tested at their maximal concentrations, whole cell (V_H=-60 mV) responses produced by AMPA (60 μM) exceeded quisqualate (30 μM) induced responses by 20-130%. In these cells, responses to either of these two agonists were usually smaller than 100 pA (V_H=-60 mV). In a second group of cells (type *B* cells; n=10), quisqualate activated currents were 5-75% larger than those induced by AMPA. In most of the type *B* cells, whole-cell responses exceeded 100 pA (V_H=-60 mV) when either agonist was applied. Kynurenate (750 μM) and CNQX (10 μM) substantially (90%) and totally blocked AMPA responses, respectively, in all cells tested (*A* and *B* types) in a voltage-independent manner. In type *A* cells, kynurenate did not substantially affect (5%) quisqualate-induced currents, while CNQX blocked 35% of the response to this agonist. In type *B* cells, kynurenate inhibited 30-60% of the quisqualate-generated response, while CNQX blocked 80% of the current elicited by this drug. It is concluded that quisqualate can bind to two sites in rat retinal ganglion cells: (1) a receptor with a high affinity for quisqualate and a relatively lower affinity for AMPA; and (2) a second site which does not recognize AMPA and has a relatively low affinity for quisqualate. Supported by an award from Fight for Sight Inc., N.Y.C. (to E.A.) and by NIH grants EY05477 and NS00879 (to S.A.L.).

318.7

EFFECTS OF EXCITATORY AMINO ACIDS AND PEPTIDES ON ACUTELY-ISOLATED SPINAL DORSAL HORN NEURONS OF THE RAT. K. MURASE, P-D. RYU, M. KANEDA*, M. RANDIC and N. AKAIKE*. Dept. Vet. Physiol. Pharmacol., Iowa State Univ., Ames, Iowa 50011 USA, and Dept. Physiol., Kyushu Univ., Fukuoka 812 Japan.

Solitary spinal dorsal horn neurons of 8-15 day-old rats were obtained by mechanical dissociation following enzyme (pronase and thermolysin, R. Gray and D. Johnston, 1987, Nature 327:620-622) treatment. The isolated cells maintained some of the morphological features, and their cell types thus could be identified.

Membrane ionic current induced by excitatory amino acids was measured by the whole-cell patch-clamp technique. At the holding voltage of -70 mV, application (0.1-100 uM for 5-20 sec) of L-glutamate (Glu), kainate, quisqualate and N-methyl D-aspartate (NMDA) produced inward current (10-1000 pA) in a dose-related manner in most of the tested cells. The response to Glu and NMDA was reduced by the presence of external Mg^{2+}, and increased by glycine (<1 uM).

Calcitonin gene-related peptide (CGRP, <1 uM) augmented the Ca current and induced a small inward shift in the holding current. CGRP also enhanced the NMDA response. Substance P (<1 uM) dose-dependently produced inward current. (Supported by JMESC, NSF and USDA).

318.8

IONTOPHORETIC MAPPING OF NMDA AND NON-NMDA RECEPTORS ON INHIBITORY AND EXCITATORY CELL TYPES OF CEREBRAL CORTEX. K.A. Jones and R.W. Baughman. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

Studies of glutamate receptors suggest many types of central neurons have both NMDA and non-NMDA receptor subtypes. In neocortex, inhibitory neurons receive excitatory input from pyramidal cells; however, it is uncertain if these neurons have both receptor subtypes. To determine the receptor profiles of inhibitory cells, neurons from the visual cortex of 7-10 day-old rat pups were dissociated and grown in culture. Intracellular voltage responses to focal, iontophoretic applications of glutamate were recorded from single cells using patch electrodes. The recording medium contained 100 uM Mg++ and 1 uM glycine to reduce the block of NMDA receptor channels at resting membrane potentials, and TTX to eliminate synaptic input. All responses to glutamate iontophoresis consisted of an NMDA-component, which was sensitive to the application of 50 uM APV, and an APV-resistant, non-NMDA component. Both presumptive inhibitory neurons, identified by their characteristically fast action potentials (McCormick and Prince, 1985; Huettner and Baughman, 1988), and excitatory neurons had non-uniform responses to glutamate applied at 20-30 um intervals along major dendritic processes. Dendritic "hot spots", 2-3 times more sensitive to glutamate than neighboring regions of dendrite, resulted from either an enhanced NMDA component, a larger non-NMDA component, or a strengthening of both components. The ratios of the two components (NMDA/non-NMDA) ranged between 1.02 to 0.34 for the cell with lowest variation, and between 4.05 to 0.15 for the cell with the highest variation. These results suggest that inhibitory and excitatory cell types have similar distributions of glutamate receptor subtypes, and that NMDA as well as non-NMDA receptors are highly localized along different regions of dendrites. Supported by NIH EY03502 and EY05810.

318.9

PHARMACOLOGY OF EXCITATORY AMINO ACID RECEPTORS IN THE RABBIT ISOLATED RETINA. A.G. Hayes* and P.J. Birch* (SPON: I. Stolerman). Dept. Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DJ, U.K.

Of importance to the study of excitatory amino acid (EAA) receptors is the use of novel in vitro preparations which permit easy pharmacological characterisation. Such a preparation is described. Californian rabbits (2-2.5kg) were killed by cervical dislocation with exsanginuation and the retina removed from each eye. A wedge-shaped strip of retinal tissue encompassing the ganglion cell fibre tract, forming the beginning of optic nerve, was dissected out and placed through a greased slot in the central barrier of a two compartment bath. Each side was superfused with Mg^{2+}-free Krebs-Ringer of the following composition (mM): KCl 4.7, NaCl 118, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 1.25, glucose 11.1 and H_2O_2 0.0024%; equilibrated with 95% O_2/5% CO_2, pH 7.4, at room temperature (18-22°C). The potential difference which developed between the two compartments was measured using Ag/AgCl electrodes and displayed on a chart recorder. Application of the EAA agonists kainate (10-100μM), AMPA (3-30μM), and NMDA (10-100μM), evoked consistent depolarisations and semi-cumulative concentration response curves could be constructed. Preliminary pharmacological results will be discussed.

318.10

NMDA-INDUCED CURRENT IN NEONATAL RAT LATERAL HORN CELLS. N. J. Dun and T. Miyazaki*(SPON: A. G. Karczmar). Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153

Lateral horn cells situated in thin transverse slices of thoracolumbar spinal cord removed from neonatal (8-16 days) rats were voltage clamped using a single electrode voltage clamp method. Pressure application of N-methyl-D-aspartate (NMDA) to lateral horn cells elicited an inward current associated with a decrease of membrane conductance at the holding potential of -30 to -90 mV. The inward current was followed in a portion of cells by an outward current associated with increased conductance. The amplitude of the outward current was generally related to the magnitude of the preceding inward current. The relationship between the amplitude of the NMDA-induced current and holding potential was V-shape, with the peak amplitude at about -60 mV in normal Krebs (1.3 mM Mg) solution. In Mg-free solution, the NMDA-induced current was substantially larger than that obtained in normal Krebs solution at all holding potentials. Further, the I-V relationship was linear in Mg-free solution. However, a decrease of membrane conductance could still be detected in a population of lateral horn cells bathed in Mg-free solution. AP5 (1-10 μM) antagonized the effects of NMDA. Cd (300 μM) consistently reduced the NMDA-induced current without affecting the holding current. The results indicate that in lateral horn cells the NMDA channels are gated by Mg ions and that influx of Ca ions is partly responsible for the inward current induced by NMDA. (Supported by NS18710).

318.11

MECHANISMS OF EXCITATION BY THE POTENT GLUTAMATE AGONIST, QUISQUALATE, IN CEREBELLAR PURKINJE NEURONS IN CULTURE. Andrea J. Yool and Donna L. Gruol, Division of Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic, La Jolla, CA.

Subtypes of the CNS glutamate receptor have been identified by three agonists, quisqualate (QA), kainate (KA) and N-methyl-D-aspartate (NMDA). Using whole-cell patch clamp, we characterized the responses to agonists in the mature cerebellar Purkinje neuron (PN) *in vitro*. The PN is insensitive to NMDA; QA is the most potent agonist. QA (0.5-2 μM), applied as a brief pulse by pressure pipette, elicited a long voltage-dependent response with several distinct components. The response was blocked by 1 mM kyurenate, but not by NMDA antagonist. The rapid Na-dependent depolarization was followed by a prolonged plateau involving both Na^+ and Ca^{2+}. Na-dependence of the initial and plateau phases was evident in Na^+-free saline (isomotic sucrose) which decreased response magnitude. The more subtle contribution of Ca^{2+} to the plateau is suggested by four lines of evidence: (1) in the absence of Na^+, QA evokes only a small depolarization; (2) in bath saline with 0.1 mM EGTA and no Ca^{2+}, the plateau is reduced in amplitude (although not blocked); (3) divalent blockers (Cd^{2+}, Mg^{2+}) reduce the plateau; (4) a Ca-dependent K^+ channel is activated during the plateau. The plateau may be mediated by cationic channels permeant to both Na^+ and Ca^{2+}. Voltage-dependence for QA was evaluated using voltage-clamp, after blocking spikes and much of the resting conductance (with TTX, TEA and Cs_{in}). The QA-activated current decreased in both amplitude and duration at hyperpolarized potentials. These properties were mimicked by KA. The prolonged excitatory responses of PNs to QA are unique, and include an increase in internal Ca^{2+}, voltage-dependence, and blockade by external divalent cations. These similarities to NMDA in other CNS regions may suggest a novel homologous role for QA in cells such as the cerebellar PN. (Supported by AA06420 and AA06665 to DLG and AA07456).

318.12

FAST DESENSITISATION OF QUISQUALATE RECEPTORS BLOCKED BY CONCANAVALIN-A IN MOUSE HIPPOCAMPAL NEURONS. M.L. Mayer and L. Vyklicky Jr*. Unit of Neurophysiology & Biophysics, LDN, NICHD, NIH, Bethesda, MD 20892.

A perfusion apparatus, consisting of an array of flow pipes each of 250 μM diameter, was mounted on a stepping motor driven manipulator, and fed via latching solenoid valves and a peristaltic pump. The solution exchange time constant was ≈ 20 msec, and produced rapid responses to agonists.

This system was used to apply excitatory amino acids to cultured hippocampal neurones under voltage clamp. Quisqualic acid produced an initial inward current response which decayed rapidly to a stable plateau. With 5 μM quisqualate the time constant of desensitisation was 72 ± 20 msec (n = 5), and the ratio of peak/plateau current 1.93 ± 0.82. The initial fast response to quisqualate recovers slowly following desensitisation, and subsequent applications of quisqualate activate only the plateau current, which shows no further desensitisation with repeated applications of agonist. The rapid nature of the initial response to quisqualate hinders its study with conventional techniques, and in our previous experiments application of quisqualate from puffer pipettes frequently evoked only the plateau response.

Pretreatment of cultures with 20 μM concanavalin-A for 10 minutes, or application of concanavalin-A to single neurones from which control responses to quisqualate had been recorded, produced an irreversible block of desensitisation. Experiments are in progress to examine the effects of lectins on responses to other excitatory amino acids.

We thank Jon Johnson for advice on rapid perfusion techniques.

318.13

EXTRACELLULAR CALCIUM PROMOTES DESENSITIZATION OF THE NMDA RESPONSE. G.D. Clark, D.B. Clifford, C.F. Zorumski. Depts. of Pediatrics, Neurology & Psychiatry, Washington Univ. Medical School, St. Louis, MO, 63110.

Studies of the regulation of NMDA responses are of importance in understanding ways to protect the nervous system from damage. Using whole cell voltage clamp techniques, we have found that rat postnatal hippocampal neurons undergo a slow process of desensitization upon exposure to NMDA and calcium. Concentrations of NMDA less than 100μM produce little desensitization. Maximal rates and percents of desensitization are seen at NMDA concentrations of 1mM. In solutions containing no added Ca^{++} (<2.5μM), very little decline in NMDA current is seen. Maximal rates of desensitization are seen at Ca^{++} concentrations of 10mM, but the percent decline continues to increase depending upon Ca^{++} concentrations. Raising intracellular Ca^{++} by activating voltage dependent Ca^{++} currents, by application of Bay k 8644, or by placing free Ca^{++} in the intracellular recording solution prior to activation or during activation of NMDA responses does not promote desensitization. The rate of decay of NMDA currents is nearly 10 times greater at membrane potentials of -50 mv versus +10 mv. The attenuation of the response is best fit by a single exponential decay. Based on these experiments we conclude that calcium acts in the electric field of the NMDA associated channel to promote desensitization, probably by an open channel blockade of the current.

318.14

EARLY AND LATE EPSP COMPONENTS IN CA1 PYRAMIDAL NEURONS FROM KAINIC ACID LESIONED RAT HIPPOCAMPUS. H.V. Wheal* and D.A. Turner (SPON: J. Davenport). Dept. of Neurophysiology, Univ. of Southampton, Southampton SO9 3TU UK.

Intracellular recordings in CA1 neurons were performed in vitro 7 days after a unilateral intraventricular kainic acid lesion. Neurons (n=4) from lesioned slices demonstrated evoked bursts of 2-4 action potentials and a late subthreshold EPSP component in response to stratum radiatum stimulation. Neither the bursts of 2-4 action potentials nor the late EPSP components were observed in control neurons (n=10). An early evoked EPSP was noted in all cells and analyzed to have a peak of 0.92±0.51 mV (mean±SD), 10-90% risetime of 8.1±3.1 ms and halfwidth of 30.9±9.1 ms (n=23 responses). The late EPSP was analyzed by subtracting an adjacent response which demonstrated an early EPSP in isolation. Such subtracted responses (n=41) showed a peak of 2.93±1.0 mV, risetime of 23.7±11.9 ms and halfwidth of of 42.9±11.7 ms. Thus, the late subthreshold EPSP component exhibited a significantly longer risetime and halfwidth than the early EPSP ($P<0.001$ by t-test).

The waveform parameters of the late EPSP component suggest a different time course of generation than for the early EPSP. Preliminary experiments also show that the late EPSP is abolished by D-APV. Thus, the kainic acid lesion may lead to the the subthreshold expression of an N-methyl-D-aspartate EPSP component in the CA1 neuron.

Supported by the Wellcome Trust and MRC (HVW); the Burroughs Wellcome Fund and a VA Research Award (DAT).

318.15

EXCITATORY AMINO ACID PHARMACOLOGY OF CA1 HIPPOCAMPAL PYRAMIDAL CELLS STUDIED BY A GREASE-GAP METHOD. M.A. Bowe, D. Martin and J.V. Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

To study the pharmacology of excitatory amino acids on CA1 hippocampal pyramidal cells, a grease-gap method was developed with use of slices that included only area CA1 and the retrohippocampal area. Longitudinal slices of 475-um thickness were cut from the caudal hippocampal formation of the rat and the regio inferior and fascia dentata were dissected away. Each slice was then transferred to a two-compartment superfusion chamber and arranged such that pyramidal cell bodies and dendrites in area CA1 were separated from their axons in the subiculum with a grease barrier. Area CA1 was exposed to 5-6 compartment volumes of each excitant concentration at 32 °C and depolarizing responses of pyramidal cells were recorded relative to their axons in the subiculum.

NMDA, AMPA, kainate and L-glutamate depolarized CA1 pyramidal cells. Tetrodotoxin (0.1 uM) little affected responses to these excitants. EC_{50} values in the absence of Mg^{2+} were 4.7, 4.6, 9.4 and 1800 uM, respectively. Phencyclidine, D-AP5 and Mg^{2+} shifted the NMDA concentration-response curve to the right in a parallel manner. pA_2 values were 5.4, 5.9 and 3.4, respectively. Similarly, pentobarbital antagonized responses to AMPA with a pA_2 value of 4.1. Schild slopes were not significantly different from unity. Mg^{2+} also reversibly reduced responses to AMPA. It appeared to be about as potent an antagonist of responses to AMPA as of responses to NMDA, but it reduced responses to AMPA by a maximum of only about one-third. Neither phencyclidine nor D-AP5 significantly altered responses to AMPA.

These results suggest the presence of a substantial NMDA receptor reserve on CA1 pyramidal cells. In addition, the activation by AMPA of the Mg^{2+}-sensitive high conductance state of the quisqualate receptor-channel appears to account for a substantial portion of its depolarizing action in this system. (Supported by NIH grant NS 16064.)

318.16

DEXTRORPHAN IS A POTENT AND SELECTIVE BLOCKER OF NMDA DEPOLARIZATIONS IN THE RAT HIPPOCAMPUS. A.E. Cole, C.U. Eccles, J.J. Aryanpur and R.S. Fisher, Dept. of Neurology, Johns Hopkins Hospital, Baltimore, MD. 21205.

Dextrorphan (DX), the O-demethylated metabolite of dextromethorphan (DM), has been shown to protect against effects of hypoxia and NMDA-mediated toxicity in CNS tissue. We examined the effect of DX on NMDA actions in rat hippocampal slices using extracellular and intracellular recording techniques. Bath application of DX (100nM-50uM) depressed NMDA-induced focal field depolarizations with an EC_{50} of 500nM. Preliminary data suggests non-competitive inhibition. DX had no effect on quisqualate-induced depolarizations. In contrast, DM showed no effect on NMDA responses at 500nM-1uM; significant block, however, was achieved with 100uM DM. Maximal block for DX and DM was reached after 60-90 min bath application and was long-lasting, with only partial recovery after 2-3 hours wash. Intracellular recordings (n=10) showed that DX (50uM) selectively blocked NMDA responses without changing RMP or input resistance. Epileptiform activity induced by bathing in zero Mg^{++} solution, an effect believed to be mediated by NMDA receptors, was blocked by bath application of 50uM DX. DX (n=3) did not demonstrate the type of use-dependent block readily observed with MK-801 (n=3). These results indicate a selective and potent action of DX to block NMDA responses in the rat hippocampus.

318.17

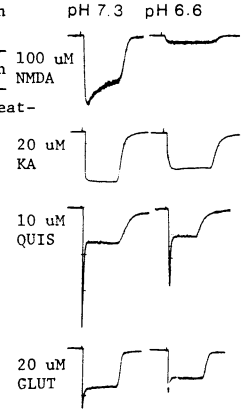
ALKALINE EXTRACELLULAR pH TRANSIENTS EVOKED BY ASPARTATE IONTOPHORESIS IN CEREBELLUM. M. Chesler & M.E. Rice. Depts. of Neurosurgery & Physiology & Biophysics, NYU Med. Ctr. 550 First Ave., N.Y., N.Y. 10016.

Electrical stimulation of parallel fibers causes an extracellular alkaline shift (AS) in the molecular layer (ML) (Kraig et al. J. Neurophysiol 49:831-50), which is blocked by kynurenate (Chesler et al. Soc. Neurosci. Abstr. 12:696). We have studied the AS evoked by iontophoresis of aspartate (Asp) in the *in-vitro* turtle cerebellum (Pseudemys scripta elegans). A pH-sensitive microelectrode and iontophoresis pipette containing 200 mM Na-Asp (pH 7.6) were secured with tips 60-90 μm apart. With superfusion of 40 mM HCO_3^--buffered Ringer, 40 sec., 100-200 nA currents produced an AS in the granule cell layer (GCL) of 0.04 ± .02 pH (range 0.03-0.07). Identical currents produced no AS in agar gel controls, or in the ML. This was true whether the assembly was lowered from the dorsal or ventral surface. In contrast, parallel fiber stimulation evoked a small AS (0.02 pH) in the ML. After lowering the buffering capacity using 10 mM HEPES Ringer, iontophoresis evoked an AS of 0.04 pH in the ML. Mn^{2+} (3-5 mM) abolished the late component of the peduncular-evoked field potential (indicating that synaptic transmission was blocked) and reduced the iontophoretically-evoked AS in the GCL by 50%. The predominance of the Asp-evoked AS in the GCL is consistent with the distribution of similarly evoked $[Ca^{2+}]_o$ and $[K^+]_o$ transients in turtle (Rice & Nicholson Soc. Neurosci. Abstr. 13:764) and may be related to the distribution of NMDA and kainate receptors which in rat, are most dense in the GCL (Greenamyre et al. JPET 233:254, 1985). (NS-10164 & NS-07745)

318.18

THE NMDA ACTIVATED CURRENT IN HIPPOCAMPAL NEURONS IS HIGHLY SENSITIVE TO $[H^+]_o$. Martin Morad* Marc Dichter and Cha-Min Tang*(SPON:E Kiclister). Dept. of Physiol. and Neurol., U of Pa., Phila., Pa.

The interactions of $[H^+]_o$ on ion channel behavior are complicated. In general acidification to pH 6.5-7.0 leads to only small suppression of channel currents. The NMDA activated current, however, shows a greater sensitivity to external pH. In superior collicular neurons Grantyn and Lux (Neuro Sci Let, in press) showed that the transient component of the NMDA current is highly sensitive to $[H^+]_o$. We found that in rat hippocampal neurons both the transient and the persistent components of the NMDA activated current were highly sensitive to acidification, Quisqualate, kainic acid and glutamate did not demonstrate this degree of $[H^+]_o$ sensitivity.



318.19

GLUTAMATE AUTORECEPTORS REDUCE EPSC's IN CULTURED HIPPOCAMPAL NEURONS. I.D. Forsythe and J.D. Clements. Lab. Developmental Neurobiology, NICHD, NIH, Bethesda MD 20892.

In cultured hippocampal neurones, fast excitatory monosynaptic transmission is mediated by kainate or quisqualate receptors while a slower monosynaptic epsp is mediated by NMDA receptors (Forsythe & Westbrook J. Physiol. 396: 515, 1988). The slow epsp is potentiated by an endogenous glycine-like substance (Forsythe et al., J. Neurosci. In press). Here we show that a fourth excitatory amino acid (EAA) receptor depresses excitatory synaptic transmission by a presynaptic mechanism.

Recordings were made from pairs of mouse hippocampal neurons grown in cell culture for 6-14 days, using whole-cell patch recording techniques. The postsynaptic cell was voltage clamped and evoked epscs recorded before, during and after application of drugs from puffer pipettes. Glutamate (1 μM) reversibly depressed the epsc in a sub-population of synaptic connections (15 were not depressed; 33 were depressed by an average of 30%). This depression was insensitive to 2-amino-5-phosphonovalerate (AP5), indicating that NMDA receptors were not involved. L 2-amino-4-phosphonobutyrate (L-AP4, 50 μM) mimicked the action of glutamate. Neither glutamate nor L-AP4 produced any detectable inward current at these concentrations. Statistical analysis of the fluctuations in epsc amplitude showed that both substances acted at a presynaptic site to reduce the probability of transmitter release. These observations strongly suggest that a fourth EAA receptor, which has a high affinity for glutamate and is distinct from kainate, quisqualate or NMDA receptors, functions as an autoreceptor in some synaptic pathways.

318.20

NOVEL RECOGNITION SITE FOR L-QUISQUALATE SENSITIZES NEURONS TO L-2-AMINO-4-PHOSPHONOBUTYRATE. Edward R. Whittemore and James F. Koerner. Dept. of Biochemistry and Neuroscience Grad. Program, Univ. of Minn., Minneapolis, MN 55455.

Brief exposure of rat hippocampal slices to L-quisqualate (Quis) sensitizes pyramidal neurons to depolarization by L-2-amino-4-phosphonobutyrate (L-AP4)(Robinson, et al., Br. Res., 381 (1986) 187-90). We report here further attempts to clarify the duration, pharmacology, mechanism, and pathway specificity of this 'QUIS-effect'.

The Quis-induced sensitization to L-AP4 decreases only 3-fold over a four hour period. No compound besides Quis has been found to induce the QUIS-effect, including Quis analogs and compounds known to stimulate second messenger systems in hippocampal slices. Of a large number of compounds assayed, only a small number have been shown to block the induction of the QUIS-effect. Although these blockers are also potent ligands at a chloride-dependent glutamate uptake site, the pharmacological profiles of QUIS-effect blockage and uptake site potency do not match. These results suggest that the QUIS-effect is induced thru a novel recognition site for Quis.

The QUIS-effect can be induced in the rat CA1, medial perforant path, and lateral olfactory tract, and in the guinea pig CA1. However, the QUIS-effect cannot be induced in the L-AP4-sensitive rat lateral perforant path (LPP), suggesting that the receptors for L-AP4 in the LPP may be distinct from those that are sensitized by Quis elsewhere. Supported by NIH NS 17944.

318.21

PERSISTENT BLOCK OF ADENOSINE ACTION IN THE DENTATE GYRUS FOLLOWING NMDA RECEPTOR ACTIVATION. K.R. Stratton, A.J. Cole, P.F. Worley and J.M. Baraban. Dept. Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Adenosine has prominent neuromodulatory actions in the hippocampus. We have recently demonstrated in the rat hippocampal slice preparation that phorbol esters which activate protein kinase C (PKC) block the effects of adenosine on synaptic transmission in the dentate gyrus. Since PKC may be invoved in long-term potentiation (LTP) following n-methyl-d-aspartate (NMDA) receptor activation, we assessed whether NMDA receptor activation also blocks adenosine action in the dentate. Bath application of adenosine inhibits the extracellularly recorded population spike (PS). Removal of magnesium from the perfusion buffer for 30 minutes results in a subsequent block of adenosine inhibition of the PS. This effect persists after re-introduction of magnesium to the perfusion buffer and is due to activation of NMDA receptors, as it is blocked by 20 μM d-2-amino-5-phosphonovalerate (APV) and does not occur if the perforant path is not stimulated during the magnesium-free period. APV does not block the action of phorbol esters. Thus, activation of NMDA receptors in the dentate gyrus results in prolonged block of adenosine action, which may be mediated via PKC activation.

318.22

EXCITATORY NEUROTRANSMISSION ACTIVATES VOLTAGE-DEPENDENT PROPERTIES IN NEURONS OF THE SPINAL MOTOR SYSTEM OF THE LAMPREY. S.T. Alford and K.A. Sigvardt. Neurology Dept., U. California-Davis, VAMC, Martinez CA 94553.

Episodes of fictive locomotion can be elicited in the in vitro lamprey spinal cord-tailfin preparation by stimulation of the tailfin. Application of strychnine to this preparation changes the pattern of locomotory activity from alternation between opposite pairs of ventral roots to synchronous activation (Alford & Williams, 1987). In this preparation ventral horn neurons demonstrate regular oscillations in membrane potential, the repolarizing phase of which does not depend on inhibitory neurotransmission (Alford & Williams, 1987). Single electrode voltage clamp studies of these neurons with CsCl-filled microelectrodes (to block outward currents that would otherwise develop at depolarized holding potentials) during episodes of rhythmic coactivation reveal inward currents coincident with ventral root bursting in the same segment. The conductance associated with the peak inward current is voltage-dependent at holding potentials depolarized from rest. The voltage dependence of this inward current is eliminated by the specific NMDA receptor blocker, APV, and by removal of Mg^{++} from the bathing solution. At very depolarized potentials a long-lasting outward current that is blocked by APV can be observed. In Mg^{++}-free saline this outward current is measurable at potentials slightly positive to rest and is also blocked by APV. These results implicate involvement of NMDA receptor activation in the generation of both the depolarization and repolarization phases of this rhythmic locomotor activity.

318.23

GLUTAMATE INHIBITS THE LOW-THRESHOLD, TRANSIENT CALCIUM CURRENT IN DORSAL ROOT GANGLION NEURONS ACUTELY IOSLATED FROM ADULT RAT. D.M. Lovinger* and F.F. Weight (SPON: S.R. Ikeda). Section of Electrophysiology, Lab. of Physiol. Pharmacol. Studies, NIAAA, Rockville, MD 20852.

Glutamate can activate receptor-coupled ion channels; however, little is known about the ability of this neurotransmitter to modulate voltage-gated ion channels. We have now observed, using isolated dorsal root ganglion (DRG) neurons, that glutamate not only activated an inward current in these cells, but also induced a relatively selective inhibition of a low-threshold, transient Ca^{2+} [Ca(t)] current. Neurons were isolated from DRGs using trypsin, collagenase and mechanical dissociation. Neurons thus isolated consisted of somata without extensive processes, making them suitable for whole-cell recording. Ca(t), studied under voltage clamp conditions in media which isolated Ca^{2+} currents, was elicited by brief jumps to a membrane potential of -35mV from a -80mV holding potential. Glutamate inhibition of Ca(t) was dose-dependent with a maximum inhibition of 70.5% at 100uM, and an IC50 of 0.7uM. Excitatory amino acid receptor agonists also inhibited Ca(t) with the order of potency: kainate > NMDA > quisqualate. Glutamate produced little inhibition of the high-threshold, sustained Ca^{2+} current (15% inhibition by 1mM glutamate), elicited by brief jumps to 0mV from a -50mV holding potential. In current clamp experiments glutamate, kainate or NMDA inhibited an afterdepolarizing potential (ADP), which was elicited by depolarizing pulses from hyperpolarized membrane potentials, and is produced by Ca(t) (G. White et al. this meeting). Since synapses onto DRG neurons are thought to occur only at axon terminals, one role for glutamate inhibition of the ADP might be to regulate terminal excitability.

318.24

INHIBITION OF EXCITATORY AMINO ACID (EAA) INDUCED NEUROTRANSMITTER RELEASE FROM RAT CORTICAL (CTL) AND HIPPOCAMPAL (HPL) BRAIN SLICES BY ω-CONOTOXIN GVIA (ω-CT). R.A. Keith*, T.J. Mangano* and A.I. Salama, ICI Pharmaceuticals Group, ICI Americas, Inc., Wilmington, DE 19897.

Recent studies have shown that EAA evoke calcium-dependent neurotransmitter release. In the present study, the release of [^{3}H] norepinephrine (NE) from CTL and HPL brain slices evoked by the EAA, N-methyl-D-aspartate (NMDA) and kainic acid (KA), was pharmacologically characterized. The effects of ω-CT, an inhibitor of neuronal L- and N-type voltage-sensitive calcium channels (VSCC), and PN 200-110 (PN), an inhibitor of L-type VSCC, were also examined. The potencies of NMDA (EC_{50}'s ∿ 100μM) and KA (EC_{50}'s ∿ 200μM) were similar in both tissues. NMDA- and KA-evoked release was ∿ 2-times greater in HPL than in CTL brain slices. Mg^{++} and MK-801, selective antagonists of NMDA receptor responses, inhibited NMDA- but not KA-evoked [^{3}H]NE release in both CTL and HPL brain slices, suggesting that NMDA and KA act at distinct EAA-receptor subtypes. Glycine did not enhance NMDA-evoked release. [^{3}H]NE release evoked by both NMDA and KA was similarly inhibited by 0.1μM ω-CT (∿25%) in both CTL and HPL slices. PN caused only a slight inhibition (∿8%) of EAA-evoked release in CTL and HPL slices. The results suggest that N-type, and to a lesser extent, L-type VSCC contribute to EAA-evoked release of [^{3}H]NE in CTL and HPL brain slices.

318.25

INTRACELLULAR INJECTION OF KAINIC ACID EXCITES APLYSIA NEURONS. G.K. Rieke and S.M. Fredman. Depts. Anatomy and Physiology, Meharry Medical College, Nashville, TN 37208.

Kainic acid (KA), an excitatory neurotoxin, is thought to act via glutamate sensitive receptors. However, EM autoradiography indicates that KA enters cells. The possibility that KA might act at intracellular sites was tested using identified neurons in the abdominal ganglion of Aplysia. KA, dissolved with L-Lysine free base and 0.25 mg/ml of carboxyfluorescein (CF), final pH ~7.0, was pressure injected into identified cells. Intracellular KA depolarized the neurons and produced a long-lasting increase (>350%) in neuronal firing. Injection of L-Lysine and L-Glutamate were without significant effect. Since extracellular application of KA also dramatically increased firing rates, neurons were examined for possible leakage using fluorescence microscopy to detect CF. In all cases the label was confined to the injected neurons and their processes. Our results show that in addition to its extracellular actions, KA also has a significant excitatory effect when acting on intracellular sites. This work was supported by NSF grant BNS-8710184 (GKR), NIH grant NS20846 (SMF), RCMI grant RR03032 and MBRS grant RR08037.

318.26

AMINO ACID ANTAGONISM OF DOPAMINE EFFECTS ON NEURONS R3-R13 OF THE ABDOMINAL GANGLION OF APLYSIA. D.G. Gibson and K.A. Marquis*. Dept. of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609.

The neurosecretory cells R3-R13 of the sea slug, Aplysia californica, were treated with 1mM concentrations of phenylalanine (Phe) and Tyrosine (Tyr), singly and in conjunction with 0.1mM dopamine (DA). DA hyperpolarized these neurons by about 7 mV; both Phe and Tyr depolarized them an average of 6 mV and caused spiking rates to increase by at least +25/min (means for Phe and Tyr not significantly different at 95% confidence). When administered with DA, Phe overpowered the DA effects and caused depolarization and spiking increases comparable to those of Phe alone. Tyr and DA together, on the other hand, caused hyperpolarization and inhibition of spiking.

Phe and Tyr compete for transport at the blood-brain barrier; high circulating levels of Phe after ingestion of the artificial sweetener Aspartame may not only impair uptake of Tyr, resulting in lowered DA synthesis, but could affect some neurons directly, if there are any neurons in the brain with the same sensitivities demonstrated in this study.

319.1

PREPULSE INHIBITION AND BACKGROUND NOISE MODULATION OF THE ACOUSTIC STARTLE REFLEX. P.K.D. Pilz* & H.-U. Schnitzler* (SPON: H.C. Diener). Dept. Animal Physiol., Univ. Tübingen, D-7400 Tübingen, FRG.

Two types of behavioral plasticity of the startle reflex have been examined in rats.

PREPULSE-INHIBITION: Startle was elicited by 10 kHz tone and broad band noise stimuli. When 100 ms prior to the startle stimulus a less intense stimulus (2.5-40 kHz, 60 dB SPL) was given - which itself does not elicit a startle response - the startle threshold was increased and the amplitude to suprathreshold stimuli decreased. The prepulse inhibition in these experiments is the more effective the more broadband or intense the startle stimulus is. Thus, the higher the activation of the auditory system, the more effective seems the prepulse inhibition.

BACKGROUND NOISE MODULATION: Amplitude and threshold of startle can be modulated by background noise. Narrow band noise (bandwidth 2 kHz, 85 dB SPL) with the center frequency beeing the stimulus frequency (13 kHz) had a masking effect. Noise with the center frequency well below (1.7 octaves) the stimulus frequency increased the startle response with little effect on the startle threshold. Noise with the center frequency well above (1.7 octaves) the stimulus frequency resulted in a decrease of the startle response and an increase of startle threshold. The high frequency noise inhibition can not be explained by simple masking, while the low frequency noise fascilitation seems to work by an enhancement of the state of the "arousal" system. Supp. by DFG: SFB 307.

319.2

BISTABLE FIRING PATTERN OF SOLEUS MOTOR UNITS DURING POSTURE IN RATS. H. Hultborn, T. Eken and O. Kiehn (Spon: J. Mogensen), Dept. of Neurophysiology, University of Copenhagen, 2200 Copenhagen, Denmark.

A bistable firing behaviour of spinal motoneurones has previously been described in the decerebrate cat (Hounsgaard et al. Progr. Brain.Res: 64, p 39, 1986). This behaviour is intrinsic to the motoneurone and generated by a plateau potential. The plateau potential causes long-lasting excitability increase and can be initiated and terminated by short-lasting synaptic excitation and inhibition.

In order to elucidate if a bistable firing pattern is present in the intact animal we have used a technique (Hennig & Lømo, Nature: 314, p 164, 1985), which allows single motor unit recording as well as nerve stimulation in freely moving rats. Most soleus motor units were tonically active for extended periods during postural activity. The distribution of interspike frequencies showed two peaks at 10-14 and 20-25 Hz respectively corresponding to two stable discharge modes. When firing at a slow tonic discharge rate maintained shifts between the two stable frequencies was initiated by short-lasting synaptic excitation (weak stimulation of the tibial nerve activating Ia afferents) or inhibition (stimulation of skin afferents). These observations demonstrate a bistable firing pattern in an intact animal during posture and suggest that plateau potentials are present under normal conditions.

319.3

SOLITARIAL DEGLUTITIVE EFFERENTS IN THE RAT. M.A. Hashim*, D. Vyas and D. Bieger. Fac. of Medicine, Memorial Univ., St. John's, NF, Canada, A1B 3V6

Efferent projections of the nucleus tractus solitarii (NTS) subnuclei containing presumptive pattern generators for swallowing were studied by means of the anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHAL). Micropneumophoresis of PHAL at glutamate-responsive pharyngeal and oesophageal NTS sites in the subnucleus intermedialis (NTSint) and centralis (NTScen), respectively, gave rise to labeling of terminals within different subdivisions predominantly of the ipsilateral nucleus ambiguus. NTScen injections produced heaviest labelling in the compact formation while NTSint injections yielded diffuse labeling in the tip of the ambiguus, and the semicompact and loose formations. In addition, a projection was labeled which terminated in the ventral and dorsal parabrachial nuclei. PHAL micro-injections placed in the nondeglutitive regions of the NTS failed to produce the pattern of labeling described above. These results demonstrate that solitarial deglutitive neurons project to ambigual divisions according to the viscerotopy delineated by Bieger and Hopkins (*J. Comp. Neurol.* 262:546-562, 1987) and, in addition, suggest a deglutitive function for the parabrachial region. (Supported by MRC).

319.4

THE EFFECTS OF AN NMDA ANTAGONIST, MK-801, ON SPINAL FIXATION IN RATS. M.F. ANDERSON*and B.J. WINTERSON. Dept. of Physiol., UNE Coll. of Ost. Med., Biddeford, ME 04005.

Electrical stimulation to a hindlimb produces a maintained flexion which persists for many days and appears to be mediated by the spinal cord ("spinal fixation"). NMDA receptors are found in the spinal cord and have been implicated in long term potentiation (LTP). Similarities between LTP and spinal fixation prompted us to examine the effects of MK-801, an NMDA antagonist, on spinal fixation.

Under anesthesia, electrical stimulation (1.75-2 mA, 7 msec, 100 Hz for 60 min) of rat hindlimb iduced a flexion which was measured by applying 0.5 g weights until stimulated and contralateral legs were equal. At 72 h, rats were anesthetized and remeasured. Then the spinal cord was transected at T7 and flexion measured.

In control rats, flexion following stimulation was 24.4 g. At 72 h, controls retained 10.4 g. After transection, hindlimb flexion increased to 14.8 g. Our prior work suggests that this increase reflects the removal of descending serotonergic inhibition. Experimental rats received MK-801 (10mg/kg, i.p.) 1 hr prior to stimulation. In these rats, flexion immediately following stimulation was 15.6 g. At 72 h, MK-801 treated rats retained 10.8 g flexion and after transection, hindlimb flexion was 9.0 g.

These results indicate that NMDA receptors are involved in the development of spinal fixation and in the circuitry affected by descending serotonergic inhibition.

319.5

STRYCHNINE ENHANCES THE FACILITATION OF THE SPINAL MONOSYNAPTIC REFLEX PRODUCED BY CONDITIONING CUTANEOUS AND MUSCLE AFFERENTS. N.A. Ibrahim and B.D. Goldstein. Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

Conditioning volleys in sural (SU) and medial gastrocnemius (MG) nerves were found to produce a biphasic effect on the monosynaptic reflex (MSR) evoked from the MG. An initial facilitation (FAC) of 28% and 60% above the control which peaked at 3 and 2 msec was followed by an inhibition (INH) of 32% and 74% below the control which peaked at 15 and 20 msec after conditioning the SU or MG nerves, respectively. Pharmacological properties of the FAC and the INH were studied by i.v. administration of strychnine (STR), bicuculline (BIC), mecamylamine (MEC) or atropine (ATR). STR potentiated and prolonged the FAC of the MSR produced by both conditioning volleys and blocked the INH produced by volleys in the SU nerve, while not affecting the INH caused by conditioning the MG nerve. Neither the FAC nor the INH produced by both conditioning volleys were sensitive to BIC, MEC or ATR. These data suggest that the FAC produced by either the SU or MG nerves would be the result of disinhibition of background glycinergic inhibitory action on motoneurons. Furthermore, the INH produced by the SU nerve but not by the MG nerve is glycine mediated. The recurrent collaterals may not play a role in the INH produced by conditioning the MG nerve.

319.6

BLOCKADE OF THE PHYSOSTIGMINE-INDUCED FACILITATION OF THE SPINAL MONOSYNAPTIC REFLEX BY A GLYCINE ANTAGONIST. B.D. Goldstein and N.A. Ibrahim. Department of Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA 30912-2300

Recent data generated in this laboratory (Ibrahim and Goldstein, Neurosci. Abs. 13:1064, 1987) have demonstrated that physostigmine (PHY) modifies the monosynaptic reflex (MSR) evoked in the L_7 ventral root of spinal cats in a dose and time dependent manner. The low dose of PHY (0.8mg/kg, i.v.) produced a facilitation lasting for three hours, whereas, the high dose (2.0mg/kg, i.v.) was found to produce an initial depression which peaked at five minutes followed by a facilitation which was maintained for three hours after the injection. In the present study we tested the hypothesis that the actions of PHY were due to the activity in the recurrent inhibitory and facilitatory pathways. Pretreatment with strychnine (0.1mg/kg i.v.) markedly antagonized the facilitation produced by the low dose of PHY and the late facilitation but not the initial depression produced by the high dose of PHY. These findings suggest that the facilitation of the MSR induced by both doses of PHY is due to disinhibition of the glycinergic inhibitory pathway. This pathway is probably a recurrent facilitatory pathway to the motoneuron. The initial depression caused by the high dose of PHY does not seem to be glycine mediated.

319.7

SPATIAL FACILITATION OF CUTANEOUS REFLEX PATHWAYS TO TRICEPS SURAE MOTONEURONS IN UNLESIONED AND CHRONIC SPINAL CATS. LaBella L A and McCrea D, Dept. Physiology, Univ. Man., Wpg, CANADA R3E 0W3.

We previously found that excitation from the caudal sural nerve predominates specifically in MG motoneurons while inhibition predominates in LG and SOL. On the other hand, effects from the lateral sural nerve are largely inhibitory throughout the triceps surae motor nuclei (TS) (LaBella et al Neurosci. Abst. 1987). The present experiments use the technique of spatial facilitation to examine the convergence of other cutaneous nerves on interneurons in these pathways. Preliminary data in unlesioned animals reveals such spatial facilitation in both the "special" excitatory sural pathway to MG and the more general inhibitory pathway to TS. Interestingly, we have also found convergence from the lateral sural pathway upon the excitatory caudal sural pathway to MG.

In studies with chronic spinal animals, sural postsynaptic potentials (PSPs) in TS were qualitatively similar to those in unlesioned cats but were greater in amplitude and had faster rises and decays (unpublished observations; see also Baker et al, Brain Res 420, 1987). One mechanism that would account for these PSP changes is an increase in the synchrony of interneuronal firing resulting in decreased dispersal of the cutaneous PSPs (Baker et al, 1987). If this is true, spatial and temporal facilitation in sural reflex pathways may be altered after chronic spinalization. Furthermore, if the apparently "special" excitatory and more general inhibitory sural pathways to TS are under different patterns of descending control, one might predict differential changes in peripheral convergence following chronic cord transection. Experiments to test these ideas are in progress. Supported by the MRC of Canada.

319.8

CHARACTERISTICS OF HINDLIMB FLEXOR PSPs EVOKED BY ACTIVATION OF Aα OR Aα AND Aδ CUTANEOUS FIBERS IN THE SPINAL CAT. J.C. Leahy* AND R.G. Durkovic (SPON: M.M. Mozell). Dept. of Physiology, SUNY HSC at Syracuse, Syracuse, NY 13210.

This report describes the characteristics of the synaptic responses of TA and PB-ST motoneurons evoked by stimulation of the cutaneous saphenous (Saph) and superficial peroneal (SP) nerves at "Aα" and "Aαδ" intensities. Experiments were performed in spinal, ischemically decapitate cats. Saph and SP were stimulated with single pulses or with trains at "Aα" or "Aαδ" intensities while recording the cord dorsum potential and the intracellular synaptic responses of TA or PB-ST motoneurons.

Saph and SP-evoked membrane potential alterations typically consisted of up to 3 phases: an early EPSP lasting 20-30ms, followed by an IPSP and then a slowly rising EPSP. During the early EPSP, motoneurons often fired a spike to both Aα and Aαδ stimulation. Increasing the stimulus intensity resulted in a decrease in central delay and an increase in the magnitude and duration of the early EPSP. Supramaximal Aα fiber activation of either Saph or SP, produced early EPSPs with a minimum central delay that averaged 2.5 ms. In a few cases a central delay of less than 1.8 ms was indicated.

We conclude that the synaptic responses elicited in TA and PB-ST motoneurons by Saph and SP stimulation are qualitatively similar for Aα and Aαδ intensities. Our data appears to be consistent with recent reports that the minimum linkage for hindlimb cutaneous reflexes may be disynaptic (see Exp. Br. Res. 69:449). Supported by NSF grants BNS 8415917 and BNS 8808495.

319.9

RECURRENT INHIBITION TO AND FROM MOTONEURONS INNERVATING THE FLEXOR DIGITORUM LONGUS AND FLEXOR HALLUCIS LONGUS MUSCLES OF THE CAT. T.M. Hamm. Div. Of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

The cat flexor digitorum longus (FDL) and flexor hallucis longus (FHL) muscles have different activities during locomotion (O'Donovan et al., J. Neurophysiol. 47: 1126, 1982), despite being anatomical and 'Ia' synergists (Fleshman et al., Exp. Brain Res. 54: 133, 1984). Fleshman et al. (1984) found no apparent differences in the pattern of recurrent IPSPs (RIPSPs) in FDL and FHL motoneurons from other motor nuclei and found no RIPSPs from FDL to other motoneurons. A larger sample of RIPSPs was obtained in this study to determine whether the distribution of RIPSPs to and from the FDL and FHL motor nuclei is more directly related to their anatomical and Ia synergism or to their function. In unanesthetized ischemic decapitate cats, it was observed that 1) RIPSPs from plantaris, medial gastrocnemius and lateral gastrocnemius-soleus (LGS) were larger to FHL motoneurons than to FDL motoneurons; 2) RIPSPs from FHL were larger to LG-S than to FDL motoneurons; 3) RIPSPs from tibialis anterior were found to FDL, but not to FHL motoneurons; and 4) no RIPSPs were observed from FDL to other motoneurons. Thus, the pattern of RIPSPs to and from FDL and FHL motoneurons corresponds more closely to function that to anatomical and Ia synergism.

Supported by USPHS grant NS22454.

319.10

THE EFFECTS OF MUSCLE FATIGUE ON SMALL DIAMETER MUSCLE AFFERENTS IN THE ANESTHETIZED CAT. L. Hayward, U. Wesselmann and W.Z. Rymer, Dept. of Physiology, Northwestern University, Chicago, IL 60611.

Recent evidence indicates that feedback from a fatiguing muscle contributes to the decline in motor unit firing rate which occurs during fatiguing voluntary contractions (Bigland-Ritchie, B., et al., J.Physiol. 378: 451, 1986). It has been hypothesized that small diameter, group III and IV muscle afferents are responsible for mediating this fatigue-related reflex since these afferents are sensitive to thermal and metabolic changes in muscle (Mense, S. & Meyer, H., J. Physiol., 363: 403, 1985). The role of these afferents in this fatigue-related reflex was evaluated by comparing the response characteristics of group III and IV muscle afferents before and during muscle fatigue.

Small diameter, slow conducting (30-1m/s) afferents originating in the triceps surae muscles were isolated and recorded from L7 and S1 dorsal rootlets in barbiturate anesthetized cats. Muscle force, length and afferent firing rate were recorded and stored on computer during muscle stretch, twitch, tetanic contraction or surface manipulation, before and during muscle fatigue. Preliminary results from 10 afferents demonstrate that muscle fatigue does induce significant increases in group III discharge rate (paired Student's t-test, $p<0.01$). 3 afferents showed significant increases in spontaneous firing rate (118-1000%) and 3 other afferents showed significant increases in discharge rate during muscle stretch (18-300%). The remaining afferents (n=5) showed increases in their discharge rates during stretch and surface pressure that were not statistically significant. There were no reductions in afferent discharge. The time course of recovery of one group III afferent (c/v 28m/s) that began discharging spontaneously during fatigue was followed for 3 minutes during which the afferent's spontaneous firing rate gradually declined until it was silent. This time course of recovery is similar to the time reported for human motor unit firing rate to recover following fatiguing maximum voluntary contractions. These preliminary data suggest that muscle fatigue does indeed modify small diameter muscle afferent responses, supporting their hypothesized role as fatigue-sensitive muscle afferents.

319.11

MEMORY SUBSTRATES IN PRIMATE SPINAL CORD PRODUCED BY OPERANT CONDITIONING OF H-REFLEX. J.R. Wolpaw and C.L. Lee. Wadsworth Labs, NYS Dpt Hlth, Albany, NY 12201.

Monkeys can gradually increase or decrease the wholly spinal, largely monosynaptic H-reflex if reward depends on reflex amplitude (J Neurophysiol 57:443-458, 1987). To define changes conditioning causes in cord, we studied reflexes after cord transection removed supraspinal control.

In 16 animals (Macaca nemestrina) the triceps surae (TS) H-reflex in one leg was increased (8 HR↑ animals) or decreased (8 HR↓ animals) by conditioning. Animals were then deeply anesthetized, and monosynaptic reflexes to L6-S1 dorsal root stimulation were recorded from TS nerve in both legs for 3 days after thoracic cord transection. Animals remained anesthetized throughout and were then sacrificed by overdose. Two effects of conditioning were clear.

First, the conditioned reflex asymmetries seen in the awake animal persisted after transection. In HR↑ animals, reflexes remained larger in HR↑ legs than in control legs. In HR↓ animals, reflexes remained smaller in HR↓ legs than in control legs. A possible source of these persistent asymmetries is plasticity at the Ia primary afferent synapse on the alpha motoneuron.

Second, after transection reflexes were small in control legs of HR↑ animals and large in control legs of HR↓ animals. Thus, removal of supraspinal influence revealed a conditioned change in the cord that was not visible in the awake animal. This effect probably reflects compensatory plasticity that allows the awake animal to maintain required background motoneuron tone while properly altering the H-reflex.

Operant conditioning of the H-reflex produces several changes in the spinal cord, several potentially accessible memory substrates. Current physiologic and anatomic studies are exploring these modifications (Lee et al., this vol). (Supp. by NIH NS22189 and United Cerebral Palsy.)

319.12

EFFECTS OF CHRONIC SPINAL HEMISECTION ON REFLEX ORGANIZATION AMONG ANKLE EXTENSORS AND FLEXORS IN CAT. JB Munson, LA Ritz, GW Sypert & TR Nichols, Dept Neurosci, U of FL Coll of Med, Gainesville, FL, and Dept Physiol, Emory Univ Sch of Med, Atlanta, GA (TRN).

Chronic spinal hemisection leads to spasticity (Carter et al., Soc Neurosci Abstr 12: 1422, 1986) and to enhanced ventral root reflexes (ibid; Hultborn & Malmsten, Acta physiol scand 119: 405, 1983) on the lesioned side. We tested muscle-specific reflexes using mechanical inputs and outputs in acute decerebrate cats, 11 mo following chronic left low-lumbar spinal hemisection. Reflexes associated with soleus (SOL), gastrocnemius (G) and tibialis anterior (TA) were measured and compared with those of control decerebrate animals (Nichols, Soc Neurosci Abstr 12: 682, 1986).

Muscle weights were normal but slightly smaller on the lesioned side. Autogenic reflexes were sometimes larger on the lesioned side, but not consistently. Inhibition from TA to SOL was observed on both sides in lesioned as in control animals, but was larger on the lesioned side. When TA was activated, inhibition from SOL to TA was weak or absent in both control and lesioned cats. The force-dependent inhibition from G to SOL was present also on both sides, but the force threshold was consistently lower on the lesioned side. These data demonstrate that the patterns of reflex interactions following lumbar spinal hemisection are similar to those of control preparations. The principal differences from normal, both on the lesioned side, are the reduced force threshold for G-to-SOL force-dependent inhibition, and the enhanced TA-to-SOL inhibitory reflex. [Supported by NS15913 (JBM), NS23683 (LAR), MRS of VA (GWS) & NS20855 (TRN)].

319.13

REFLEX ORGANIZATION AMONG ANKLE EXTENSORS AND PRETIBIAL FLEXORS IN THE CHRONIC SPINAL CAT. T.C.Cope and T.R.Nichols Dept. of Cell Biology, Univ. of Texas Health Sciences Center, Dallas, TX 75235 and Dept. of Physiology, Emory Univ., Atlanta, GA 30322 (TRN).

The mechanisms underlying the striking changes in spinal reflexes and muscle tone observed long after complete transection of the spinal cord remain unclear. To assess the involvement of both muscular and neural components, we measured muscle-specific reflexes associated with soleus (SOL), gastrocnemius (G) and tibialis anterior (TA) muscles in terminal experiments conducted up to 11 months following low thoracic transection. Weights of TA and G were within normal limits while SOL was atrophic and pale in color. When SOL was electrically stimulated, the prominent stretch-evoked yielding behavior exhibited by normal solei was not observed. Autogenic reflex strengths were within limits for control decerebrate cats, but background force and reflex stiffness were highly variable. No consistent asymmetries in autogenic reflexes were noted beteeen homologous muscles on the two sides. The reciprocal inhibition normally observed from TA to SOL was weak or absent, and heterogenic inhibition from G to SOL, while observed in averaged responses, was not statistically significant. These results support earlier suggestions that a reduction of central inhibition contributes to reflex abnormalities in cats with transected spinal cords.

(supported by NS20855 to TRN and NS21023 to TCC)

319.14

THE EFFECTS OF POSTSYNAPTIC INHIBITION ON THE MONOSYNAPTIC REFLEX OF THE CAT. **Charles Capaday* and Richard B. Stein, Department of Physiology, The University of Alberta, Edmonton, Canada T6G 2H7.**

Results of a computer simulation study (Capaday and Stein, J. Neuroscience. Meth. 21:91-104, 1987) revealed that presynaptic inhibition of the Ia-afferents projecting to a motoneuron pool was the only central mechanism that could control the size of the monosynaptic reflex when comparisons were made at the same level of recruitment. In our model, the extra excitatory current necessary to restore the recruitment level to the desired value also restores the size of the monosynaptic reflex response. We have, therefore, tested the prediction concerning the effects of postsynaptic inhibition in postmammilary precollicular, decerebrate cat. Activation of the soleus motoneurons was produced by stimulation of the contralateral common peroneal nerve or by spontaneous episodes of motor activity. The monosynaptic reflex was elicited by stimulation of the cut L7-S1 dorsal roots. Tonic post-synaptic inhibition of the soleus motoneurons was produced by activation of Renshaw cells through repetive antidromic stimulation of the LG-MG nerves. When the amplitude of the monosynaptic reflex is plotted against the isometric soleus tension, a measure of the recruitment level, the data obtained when the motoneurons were under the influence of postsynaptic inhibition falls along the same straight line as the data obtained in the control condition. Presynaptic inhibition, on the other hand, can control the amplitude of the monosynaptic reflex independently of the recruitment level.

319.15

MECHANISM OF YIELD COMPENSATION IN REFLEXIVELY ACTIVE MUSCLE DURING RAMP STRETCH. Di. Boškov* and W. Z. Rymer, Dept. of Physiol., Northwestern Univ., Chicago, IL 60611. The mechanical output of reflexively active skeletal muscle demonstrates grossly linear, springlike behavior in response to ramp and hold stretches, whereas the stiffness of areflexive electrically activated muscle falls abruptly (a condition called "yield") after the amplitude of stretch exceeds a fraction of a millimeter. The patterns of spindle afferent discharge and the subsequent increase in motoneuronal activity, manifested as increase in EMG, strongly suggest that these reflex components are primarily responsible for linearization of muscle output. However, the fact that the reflex component of EMG occurs before or near the time of yield (depending of stretch velocity) indicates that some form of predictive mechanism is involved. In this study, we modeled the behavior of muscle under a variety of activation conditions.

Computer simulations of muscle responses, based on Huxley's model with Zahalak's modification, have shown that yield can be prevented by recruiting new motor units in order of increasing size, starting shortly after the onset of stretch. Subsequent recruitment of new units improved linearity during the later phase of stretch. Step increase in motoneuronal discharge of already recruited units prevented yield to some extent, but responses during the later phase of stretch were less linear. Based on these and previous results obtained during electrical stimulation of cat triceps surae muscles (which suggested that the yield is most effectively prevented by recruiting more motor units after the onset of stretch), we produced a model of motoneuron pool. This model consisted of a group of threshold devices, each representing a group of motoneurons with similar firing thresholds in order of increasing size, with common input in form of integrator of motoneuronal input. To complete the reflex loop, we used a nonlinear model of spindle response whose output was proportional to the low power of stretch velocity. Simulations of the whole reflex loop, including conduction delays, yielded responses in good agreement with well known responses of reflexively active muscle. These results support the hypothesis of preferential role of early recruitment in yield compensation and reinforce the significance of predictive or anticipatory regulation of muscle mechanical properties by reflex pathways.

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319.16

EFFECTS OF DIFFERENT SYNAPTIC INPUTS ON THE DISCHARGE PROPERTIES OF A MODEL POOL OF ALPHA MOTONEURONS. R.K. Powers, Dept. of Physiol. & Biophys., Univ. of Wash., Seattle, WA 98105

Acute partial spinal lesions produce a number of alterations in the discharge properties of cat medial gastrocneumius (MG) alpha motoneurons, including a decrease in minimum steady discharge rates and an increase in discharge synchrony (Powers and Rymer, Soc. Neurosci. Abstr. 12:465,1986). These alterations could result from changes in the pattern and magnitude of synaptic inputs to the MG pool. A computer model of a small portion of the MG motoneuron pool (20 cells) has therefore been developed to investigate the effects of different patterns of synaptic input on motoneuron discharge behavior.

The neuron model used is a modification of that described by MacGregor and Oliver (Kybernetik 16:53, 1974). Currents flowing through synaptic and spike activated conductances are summed and divided by the total cell conductance to determine their net effect on membrane potential. Spikes occur whenever this value exceeds a set threshold and are followed by exponentially decaying potassimum (K) and calcium (Ca) conductances. Values of voltage threshold, input conductance, maximal K and Ca conductances and time constants of decay were chosen so that discharge behavior matched that described for type S and FR MG motoneurons.

Preliminary simulations have focussed on the effects of Renshaw inhibition on motoneuron discharge behavior, and have indicated such effects to be rather weak. The ability of common excitatory input to synchronize motoneuron discharge is slightly reduced in the presence of high levels of Renshaw inhibition, but minimum steady discharge rates are unaffected. The dependence of motoneuron discharge behavior on the magnitude, distribution and spectral content of other common excitatory and inhibitory inputs to the pool is currently being investigated.

(Supported in part by NIH grant #NS25206.)

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM II

320.1

COMMISSUROTOMY IMPAIRS BIHEMISPHERIC COORDINATION OF VISUALLY GUIDED PURSUIT EYE MOVEMENTS. E.G. Keating. Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, N.Y. 13210.

Some eye movements require cooperation between the two cerebral hemispheres. For example, if a visual target appears in the right visual field and moves away from the fovea, the left hemisphere computes the retinal velocity of this target, but the motor command for rightward smooth pursuit comes primarily from the right hemisphere.

Some of this interhemispheric cooperation occurs at the level of the forebrain. Two monkeys learned to pursue step-ramp targets that appeared in the left or right visual field and moved toward or away from the animal's fixation point. The posterior half of the corpus callosum was then transected. The surgery slowed pursuit for a period of one to three weeks. Eye acceleration and peak velocity during the initial 100 ms of pursuit and also peak velocity during the maintenance phase of pursuit were abnormal. In at least one of the monkeys the deficit cannot be laid to generalized trauma, since only pursuit of targets moving away from the fovea was significantly impaired. (NSF BNS8603915).

320.2

BRAIN REGIONS IN HUMANS ACTIVATED DURING SMOOTH PURSUIT VISUAL TRACKING. F. Miezin*, C. Applegate*, S. Petersen* and P. Fox, (SPON: T.O. Videen) Caltech, Pasadena, CA 91125 and Wash. Univ. Sch. of Med., St. Louis, MO 63110.

Positron Emission Tomography (PET) was used to monitor cortical neuronal responses as reflected by increased cerebral blood flow while subjects followed a visual target. Three horizontal target motions were used: sinusoidal; constant velocity to the right with return saccade to the left; and constant velocity to the left, return to the right; and a control state where the target is stationary. Two sets of scans for each target motion were obtained during a single session from each subject (n=7). The control scan was subtracted from each of the activated scans, and the resulting difference images were averaged for each of the stimulation conditions.

The cortical areas activated during pursuit eye movements include a dorsal area in parieto-occipital cortex (PO); a lateral area in temporo-occipital cortex (TO); and a region including primary and adjacent visual cortex. Horizontal saccades elicit responses in PO, the primary and secondary visual areas, the supplementary motor area, cerebellum and the frontal eye fields. No responses were seen in TO. Area TO is near a motion-processing region that may be homologous to area MT in macaques (ARVO abstract, 1988). Since TO is activated during pursuit eye movements and is near the MT homologue, TO may be homologous to area MST, a region in macaque cortex active during pursuit eye movements.

320.3

PROJECTIONS FROM THE FRONTAL LOBE EYE FIELDS TO THE UPPER BRAIN STEM IN MONKEY. B.L. Shook, M. Schlag-Rey and J. Schlag. UCLA, Dept. Anatomy and BRI, Los Angeles, CA 90024

Do patterns of connectivity of the supplementary eye field (SEF) more closely resemble those of the arcuate frontal eye field (FEF) or other cortical motor regions ? WGA-HRP (10%; TMB histochemistry) was used to study pathways of the SEF, FEF, SMA and primary motor cortices. In 2 monkeys the SEF was defined via recording and stimulation methods.

Only SEF and FEF injections labeled pre-oculomotor nuclei. SEF as FEF projects to the ipsilateral pretectum (n. limitans and n. optic tract) and the interstitial n. of Cajal. All injections labeled the superior colliculus. SEF projects to layers I, III and IV and FEF to I-IV. SMA projects to ventral IV and motor cortex to VI. Sparse pathways were found from SEF to the raphe omnipause region and from FEF to n. olivary pretectalis.

All areas injected project to the basis pontis but the terminal nucleus varied according to the locus of the injection (e.g. FEF alone to n. dorsolateralis). The pontine tegmentum exhibited dense anterograde label in n. reticularis tegmentis pontis; dorsal injections labeled the medial, and lateral injections the lateral region of this nucleus.

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320.4

RECOVERY OF EYE MOVEMENTS AFTER BILATERAL FOVEA LESIONS IN MONKEYS. S. J. Heinen* and A. A. Skavenski. Dept. Psych., Northeastern Univ., Boston, MA 02115.

This report is concerned with the characteristics of fixation and saccadic eye movements in monkeys treated by laser photocoagulation to simulate bilateral human macular degeneration. The results come from a broader study of the behavioral and neural changes that accompany this major cause of blindness. Discrete time and location of lesions uncovered differences between the eye movements not seen in disease. Monkeys were trained to fixate a small target and track it with saccades when it was stepped to randomly selected 10 deg arc eccentricities. Just after lesions, animals formed a new preferred retinal locus (PRL) where fixation was stable. This PRL did not change position over long time periods. Just after lesions, primary saccades placed eccentric targets on scotomatous fovea. Small corrective saccades moved targets to the PRL. Over a period of several months, slowly increasing percentages of primary saccades directed targets to the new PRL or other intact retina. Thus, both oculomotor systems were plastic, but the saccadic system appeared much more difficult to reprogram.

320.5

VISUAL MOTION PROCESSING IN HUMANS AFTER LOSS OF STRIATE CORTEX. L. Tychsen, M. Rizzo, R.R. Hurtig*, K.W. Stevens*, E.J. Engelken*. USAF Sch Aerosp Med, Brooks AFB TX 78235.

The striate cortex provides the major input to motion processing areas of extrastriate cortex in primates. A minor input to extrastriate cortex may also be provided directly from subcortical structures. The minor input might be evident as a weak capacity for motion processing after loss of striate cortex.

We tested motion processing after loss of striate cortex in 2 patients who had unilateral occipital lobectomies 3 and 10 years before entry. Tissue loss was documented by CT and was evident behaviorally as an absolute hemianopia. Motion processing was measured as transient pursuit eye movement evoked in 750-1500 individual trials of ramp stimulus motion, using a scleral coil to record eye movement. Motion of a high luminance spot (0.5 deg, 1400 fL He-Ne laser) at eccentricities 3-30^{o} within the hemianopic field evoked pursuit in the appropriate direction. Latencies were approximately twice as long, and pursuit accelerations one-fifth to one-half as robust, as those evoked by equivalent motion in the sighted field. Step changes of spot position in the hemianopic field did not evoke accurate saccades. Motion of a large pattern (luminance 2.5 fL) in the hemianopic field failed to evoke optokinetic nystagmus.

Our findings indicate a weak capacity for visual motion processing in humans after loss of striate cortex. It is possible that this capacity is mediated by minor inputs to extrastriate areas from structures such as colliculus.

320.6

OCULAR MOTOR DEFICITS IN RETINOTOPIC AND CRANIOTOPIC SPACE IN MONKEYS WITH UNILATERAL STRIATE AND CORPUS CALLOSUM LESIONS. R.J. Tusa, S.J. Herdman, and M. Mishkin. Johns Hopkins University, Baltimore, MD 21205, and Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

We examined the role of visual input from striate cortex (V1) to one cerebral hemisphere in the generation of saccades. Three rhesus monkeys with a scleral coil were trained to fixate a target light randomly stepped to 1 of 9 positions between L20° & R20°. 2 monkeys then had a one-stage unilateral V1 ablation and section of the posterior half of the corpus callosum (CC), while the third monkey had a unilateral V1 ablation followed 6 weeks later by a CC section. We tested the animals for 6-12 months post-op.

Retinotopic space. During the first 2 post-op weeks, the monkeys did not make visually-guided saccades to dim (3 ft-L) or bright (2000 ft-L) lights in the visual field contralateral to the V1 lesion. By 1 month, they made visually-guided saccades to bright and dim lights in the defective visual field, but they did not recover the ability to make express saccades (latency < 100 msec). These defects were unrelated to the craniotopic location of the target.

Craniotopic space. During the first post-op week, the 2 monkeys with the one-stage operation did not make spontaneous or target-searching saccades into craniotopic space contralateral to the V1 lesion. By 1 month this deficit resolved, but the latencies for visually-guided saccades increased progressively for targets located further in contralateral craniotopic space. The monkey with the isolated V1 lesion had similar deficits after the CC section.

In summary, we found retinotopic and craniotopic saccade deficits following unilateral V1 + CC lesions. Within 1 month of a V1 ablation, other areas can mediate the retinotopic location of visually-guided but not express saccades. The deficits related to craniotopic space are more persistent, and may be due to defects in directing visual attention, constructing an internal map of extrapersonal space, or both.

320.7

RELATIONSHIP BETWEEN CATCH-UP SACCADE FREQUENCY AND SMOOTH PURSUIT GAIN IN SCHIZOPHRENICS AND NORMALS. LA Abel, L Friedman, J Jesberger* and HY Meltzer, Biomed. Eng. Dep't, U of Akron, Akron Ohio, 44325, and Psychiat. Dep't, CWRU and University Hospitals of Cleveland.

One approach used in evaluating pursuit has been counting catch-up saccades (CUS). Since these are generated in response to a position error arising from low-gain pursuit, they seemingly should correlate with pursuit gain. But, if there is a variation in how much position error is tolerated before generation of a CUS, the same number of CUS might occur with considerably different gains. We tested 10 schizophrenics and 10 normals, plotting their pursuit gains versus number of CUS, for 3 cycles of tracking at 5^{o}/s. There was a statistically significant correlation between CUS and gain in normals (-.61, p<.05, Pearson r), but not for schizophrenics (-.03). This suggests that the neural mechanism which generates corrective saccades is less consistent in schizophrenics than normals. Some patients may have a lower threshold for position error correction. This is in contrast with the apparent increase in the velocity error threshold seen in the same group of patients (Friedman et al., this volume). CUS counting may not be a good reflection of pursuit gain in these patients; the results also suggest differences in error processing between schizophrenics and normals. Supported in part by USPHS Grant # MH41684.

320.8

INCREASE OF HYPOMETRIC SACCADES IN SCHIZOPHRENICS
A.Mackert*, C.Woyth* and M.Flechtner*
(SPON: J.Kasper). Department of Psychiatry, Free University of Berlin, 1000 Berlin 19 (West) F.R.G.

Dysfunctions of eye movements are believed to be a possible psychophysiologic correlate of schizophrenia, best known are dysfunctions of the smooth tracking system. We investigated the accuracy of saccadic eye movements in 47 schizophrenic patients having acute psychotic symptoms. Diagnostic criteria fulfilled RDC-requirements for schizophrenia. Psychopathology was assessed with the Brief Psychiatric Rating Scale, the Scale for Assessment of Negative Symptoms, and the Prognostic Scale. No patient was treated with neuroleptics at the time of the examination or showed neurological abnormalities. The age range was 17-57 years. Horizontal eye movements were recorded with DC-Electro-oculography. The patients were given the task to fixate a target that was displaced horizontally at unpredictable intervals and at random amplitudes (7.5 to 20°) in both directions.

Schizophrenics produced significantly more dysmetric, undershooting saccades than a control group of sex- and age-matched healthy volunteers. Undershooting was especially prominent for saccades with small amplitudes. The prevalence of dysmetric saccades was significantly correlated with the length of illness, poor prognosis, and motor retardation. This impairment of visuomotor performance is more likely caused by dysfunction of cortical than by subcortical structures.

320.9

NEW ASPECTS OF DEFECTIVE PURSUIT IN SCHIZOPHRENICS

L Friedman, IA Abel, J Jesberger*, HY Meltzer, Psychiat. Dep't, Case Western Reserve U and University Hospitals, Cleveland, Ohio 44106, & Biomed. Eng. Dep't, U of Akron.

There have been few quantitative studies of smooth pursuit eye movements of schizophrenics. We examined the tracking of 5°/s targets by neuroleptic-treated schizophrenics (N=10) and normals (N=10), measuring time- weighted pursuit gain, catch-up saccades (CUS), mean duration of tracking segments, linearity of those segments (average Pearson r) and right/left gain ratio.

Mean gain for the schizophrenics was lower (.81 vs .99, p<.001, Mann-Whitney U) in agreement with previous reports. CUS were also more numerous (26.2 vs 9.6, p<.002) and mean segment duration lower (450 vs 710 ms, p<.001) for 3 cycles of tracking in the schizophrenics. Both groups had symmetric (left vs right) pursuit.

The most novel finding was the significantly poorer linearity of the pursuit segments made by the schizophrenics (.947 vs .989, p<.002). Thus, tracking deviated around a straight line without causing sufficient position error to elicit a saccade. This suggests that control of eye velocity is poorer in schizophrenics. Thus, schizophrenic pursuit tracking is grossly defective as measured by average gain. It is also defective in fine control as measured by linearity. Supported in part by USPHS Grant # MH41684.

320.10

SPONTANEOUS SACCADES IN THE PIGMENTED RAT FOLLOWING INFERIOR OLIVE AND CEREBELLAR LESIONS.

P. Strata*, L. Chelazzi*, M. Ghirardi*, F. Rossi* and F. Tempia* (SPON: European Neuroscience Association). Dept. of Human Anatomy and Physiology, University of Turin, 10125 Turin, Italy.

Rat saccadic eye movements have been chosen as a model to investigate the effects of inferior olive (IO) and cerebellar lesions on motor behavior. The horizontal component of spontaneous saccades has been recorded by a coil system in 20, head-restrained, pigmented rats. 5 animals served as controls. In 5 rats the IO was bilaterally destroyed, at least 1 month before recording, by means of 3-acetylpyridine. In 5 other rats the posterior vermis (lob. VI-IX) was ablated. The flocculus and paraflocculus were bilaterally removed in the remaining 5 rats. No clear saccadic impairments were observed in vermectomized animals. On the contrary, following either IO or flocculo-parafloccular lesion, saccades were characterized by a large, backward post-saccadic drift with an exponential time course (time constant 150-200 ms). Careful analysis did not reveal any significant difference between the effects of these two lesions. Such a drift is due to a mismatch between the phasic and the tonic components (the pulse and the step) of saccadic innervation with a significant reduction of the latter. Our results show that, in the rat, the flocculus and/or paraflocculus together with the IO are essential to properly coordinate the phasic and tonic activities in saccadic eye movements.

320.11

INFLUENCE OF TEMPERATURE ON OPTOKINETIC AND VESTIBULAR EYE MOVEMENTS IN GOLDFISH.

J. G. McElligott, M. Weiser* and R. Baker (SPON: J. O'Neill). Dept. Pharmacol., Temple Univ. Sch. of Med., Philadelphia, PA 19140 and Dept. Physiol. & Biophys., NYU Med. Ctr., New York, N.Y. 10016

Temperature acclimation must be requisite for appropriate motor behavior in ectothermic vertebrates. This hypothesis was examined for visual-vestibular control of horizontal eye movements and adaptive VOR gain changes over a temperature range of 4-21°C. Goldfish acclimated to 21°C for two months were tested with sinusoidal visual and vestibular stimuli (0.032-5.0 Hz) at 16-32°/sec. Little alteration was observed in the eye movements recorded until temperature reached 11°C; however, gain modifications after visual-vestibular interaction were already significantly reduced by 16°C. Further cooling from 11 to 4°C gradually decreased both optokinetic and vestibular eye velocity as well as the ability to either suppress or increase the gain of the VOR proportional to stimulus frequency. Around 6°C the VOR noticeably decreased in amplitude; however the phase of eye velocity remained constant. Close to 4°C, stable eye position and spontaneous saccades were still observed, but the VOR exhibited nearly a complete loss of fast phases. All temperature-related effects were reversible. These data demonstrate that compensatory visual and vestibular control of eye movement, except for adaptive gain control, rapidly adjusts to large fluctuations in temperature. Supported by EY02007 and NS13742.

320.12

OPEN LOOP OPTOKINETIC NYSTAGMUS OF THE SQUIRREL MONKEY EVOKED FROM A BoTx-IMMOBILIZED EYE.

O.-J. Grüsser and F. Behrens*, Dept. of Physiology, Freie Universität, Arnimallee 22, 1000 Berlin 33, Germany

Botulinum toxin (BoTx "Oculinum", San Francisco 10-11 units) injected into the retrobulbar tissue of Squirrel monkeys (Saimiri sciureus) induces within 2 to 3 days a complete immobilization of all extraocular muscles lasting for about 10 to 12 days and recovering thereafter to normal mobility of the eye within another 10 days. This technique provides a simple method to study the open loop optokinetic nystagmus (OKN). Eye movements were recorded by means of the electromagnetic search coil technique and the recordings were performed in awake squirrel monkeys.

1) In normal squirrel monkeys gain of horizontal OKN reached values between 0.8-0.97 at stimulus angular velocities below 150 $deg.s^{-1}$ (2.37 or 15° black and white vertical stripe pattern).

2) Optokinetic stimulation of the immobilized eye led to vigorous OKN and OKAN of the other eye, whereby maximum gain was found at low retinal stimulus velocities V_r (2-5 $deg.s^{-1}$, gain above 30).

3) Increasing V_r led to a decrease in gain with a slope of about -20 dB/decade.

4) When a stripe pattern suddenly appeared, rotating at a constant angular velocity, the open loop OKN-gain increased with time and reached a maximum about 40-50 seconds after stimulus onset. Thus, open loop OKN-gain is a time dependent value.

The work was supported in part by a grant of the DFG (Gr 161).

320.13

SENSORY AND MOTOR EFFECTS OF MONOCULAR DEPRIVATION ON THE OCULOMOTOR RESPONSES IN SQUIRREL MONKEYS.

F. Behrens* and O.-J. Grüsser. (SPON: European Brain and Behavior Society). Dept. of Physiology, Freie Universität Berlin, Arnimallee 22, D-1000 Berlin 33, Germany

By means of the electromagnetic search-coil technique the optokinetic nystagmus was measured in three normal and four monocularly deprived adult squirrel monkeys. Monocular deprivation had been achieved by lid suture performed during the first 10 days of life. The duration of lid closure varied between 1.5 and 9 years. Some weeks before commencing the experiments the eyelids were opened and the lid canthus reconstructed.

Normal animals responded to horizontal optokinetic stimulus velocities up to 350-400 $degrees.s^{-1}$ (vertical stripe pattern 2.4 or 15° period). OKN-responses to monocular and binocular stimulation showed only slight differences in the upper velocity ranges and. no directional selectivity. In monocularly deprived animals OKN aroused by monocular stimulation of the non-deprived eye was similar to that in normal animals. Optokinetic stimulation of the deprived eye evoked an OKN with a reduced gain and significantly smaller maximum angular velocity as well as a much higher variability in the responses compared to the OKN evoked through the normal eye. Some directional differences (temporo-nasal/naso-temporal) were found.

Since all monocularly deprived animals exhibited squinting of the deprived eye - preferentially towards the nasal side - monocular deprivation also has an effect on the "efferent" components of the oculomotor system.

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320.14

THEORETICAL MODELS AND COMPUTER SIMULATIONS OF THE OPTIC TECTUM OF THE BARN OWL.

J.C. Pearson*, C.D. Spence*, W.E. Sullivan, J.J. Gelfand* and R.M. Peterson*. SRI-David Sarnoff Research Center, CN5300, Princeton, NJ 08543; Dept. of Biology, Princeton Univ., Princeton, NJ 08544.

The optic tectum of the barn owl contains a map of space that registers the positions of visual/acoustic stimuli and encodes the head saccade vector needed to center the stimuli in the field of view. The sensory maps in the tectum derive from topographic projections from purely visual and acoustic maps in the retina and the external nucleus of the inferior colliculus (ICX), respectively (J. C. Neurol. 218). In young owls, loss of visual/acoustic registration produced by prisms or ear plugs is corrected by compensatory shifts in the acoustic map if visual experience is provided (Science 230). This shift could be caused by changes in the tectum itself, or could simply reflect changes in the earlier stages that produce the ICX map. Our model tests the first possibility. We assume (predict) that the ICX-to-tectum projection is topographic and anatomically broad, but is functionally sharpened through synaptic plasticity. Synapses are strengthened (weakened) if the pre-synaptic element is active (inactive) and the post-synaptic voltage is greater than the threshold value, Ts (Tw). The important parameters in the model are the values of Ts and Tw relative to the strength of the fixed visual connection strength, Cv (e.g., Tw > Cv > Ts). Fusion itself can be achieved with many different choices for these relationships, however each choice is distinguished by the way the connection strengths and the receptive fields (RFs) change in time, and by the maximum correctable separations between the visual and acoustic RFs. These maximal displacements are independent of the quadrants the RFs are in, thus calling for a re-examination of the "quadrant limit" effect (Science 230). The model is also in disagreement with extracellular HRP tracing studies that show the ICX-to-tectum projection to be fixed and point-to-point. However, if the plasticity does occur at earlier stages of acoustic processing, as this evidence suggests, it is unclear how visual experience could control it, since these stages are not known to receive direct visual input.

Recent experiments with two stimulating electrodes show that the head saccade made in response to simultaneous stimulation is roughly the average of the response made to each individually (Soc. Neuro. Abstr. 1987 112.10). We present two models of how the average positional information encoded on a neural space map could be computed. One model employs a quadratically weighted excitatory topographic projection onto another map with local lateral inhibition. The other employs linear gradients of excitatory connections of opposite slope onto separate motor pools that set the effective spring constants of the agonist/antagonist muscle groups that rotate the head.

(Supported by internal development funds of the David Sarnoff Research Center.)

320.15

A MODEL OF THE SMOOTH PURSUIT SYSTEM WITH REALISTIC EMERGENT PROPERTIES. R.J. Krauzlis* and S.G. Lisberger. Dept. of Physiology, Div. of Neurobiology, University of California, San Francisco, CA 94143

The primate smooth pursuit system uses visual motion information to initiate and guide tracking eye movements. Lesion and electrophysiological studies have identified several brain regions that are involved in this behavior, but it is difficult to draw conclusions about their roles, because the actual signals used by the pursuit system are not known. To assess the role of particular visual motion signals, we have used behavioral data as the basis for a computer model of the pursuit system.

We measured the initiation of pursuit in Rhesus monkeys using the scleral search coil technique. Since analysis of the first 200 ms of pursuit indicates that there are at least three components in the response to moving targets, the model contains three parallel visual pathways. The first pathway is sensitive to image velocity, the second to image acceleration, and the third to the impulse of image acceleration that accompanies the onset of target motion. The model was tuned by adjusting each pathway so that the rising phase of the response to constant velocity and acceleration targets matched the data obtained from monkeys. The model does not explicitly reconstruct target velocity; instead, eye acceleration is driven directly by different aspects of visual motion. The model has several interesting emergent properties. First, the closed loop behavior of the model closely matches closed loop data, even though the model was based strictly upon the rising phase of open loop data. Second, the model shows steady-state oscillations of the same frequency as seen during sustained tracking in the monkey. Driving either the model or the monkey's pursuit system at this resonant frequency results in a 180 degree phase shift between eye and target. Finally, increasing the delay of visual feedback in the model increases the period of oscillations just as in the experimental data. The corroboration of these emergent properties by experimental data indicates that the elements within the model capture important aspects of signal processing within the smooth pursuit system. (Supported by NIH grant EY03878)

320.16

THE LINEARIZING PROPERTIES OF BILATERAL STRUCTURES IN THE OCULOMOTOR CONTROL SYSTEM. H.L. Smith* and H.L. Galiana. Biomed. Eng. Unit, McGill University, Montreal, Que. Canada H3A 2B4.

We have explored theoretically and through model simulation, the linear operating range of bilateral motor circuits in the brainstem, controlling eye movements. We considered the vestibulo-ocular reflex (VOR), which causes conjugate eye movements opposite to angular head rotation, during the slow-phases of vestibular nystagmus. Its linear range is evaluated from the ratio peak-eye-velocity/peak-head-velocity during step or sinusoidal head rotations. In the VOR's three-neuron arc, second order neurons in the vestibular nuclei typically have half the resting rate and at least twice the sensitivity of primary vestibular neurons. Hence, the VOR should be non-linear at head velocities well below the linear range of the canal (1/4 of canal range in this example). Yet, behaviourally, it remains linear for rotations beyond the linear range of both central and primary neurons!

Simulations of a bilateral model of the VOR [JNP 51:210,'84] show that a very simple mechanism could account for the large linear range of the VOR, and possibly of all motor systems. The use of a bilateral circuit introduces two aspects to central responses: The first causes reciprocal central modulations, in response to the difference between sensory or descending inputs on the two sides, which would eventually silence neural pools on one side. The other responds to the sum of bilateral inputs and raises equally the activity on the two sides, i.e. raises the 'background resting rate' dynamically. Analysis and graphs show that these two components complement each other to linearize the canal information and avoid central cut-offs. As a result, the observed linear range of a reflex can grow automatically, or extend itself, with increasing amplitudes on the sensory inputs. This property would interact with the additional, beneficial, effects of nystagmus reported earlier [SNA 12:297.16,'86].

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320.17

SHORT TERM POTENTIATION AS A MECHANISM FOR A CENTRAL INTEGRATOR L. Shen* (SPON: R. Poppele) Lab. of Neurophysiology, Univ. of Minnesota, Minneapolis, MN 55455

Neurophysiological studies in oculomotor systems suggest that a time integral operation is performed by neural circuits. Previous models of the neural integrator incorporate positive feedback to generate the required time constants. Such models are generally unsatisfactory due to stringent parameter requirements and inherent instability. A new model using presynaptic potentiation phenomena is proposed. It is shown that under certain conditions, the nonlinear relation between presynaptic input and postsynaptic output has the property of an integral operator. Unlike previous models, this model is robust and without unstable singular points. Fluctuations of the parameters involved account naturally for the observed variations in the behavior of neurons in the literature. Possible incorporation of the model in the context of the vestibular-oculomotor reflex is discussed. It is shown that the model fits within the three neuron pathway. The eye position signals commonly observed in the vestibular neurons can now be explained by synaptic interactions without the extra pathways usually assumed. Results of lesion studies on cerebellum and brain stem are also accounted for by the model. It is emphasized that the nonlinear synaptic interactions between neurons have potentially very powerful computational capacity.

Supported by grant BNS 8518714 from the NSF.

SENSORY SYSTEMS: AUDITORY SYSTEMS VI

321.1

AUTOREGULATION OF COCHLEAR BLOOD FLOW IN WISTAR-KYOTO RATS. W.S. Quirk, J.K. Coleman, J.W. Wright and H.A. Dengerink. Dept. of Psychology, Washington State Univ., Pullman, WA 99164-4830.

Previous investigations in our laboratory observed changes in cochlear blood flow (CBF) in normotensive Wistar-Kyoto (WKY) rats that could be interpreted as autoregulation of CBF. To test this hypothesis WKY rats were intra-arterially infused with high doses of phenylephrine (0.02 mg/kg/min in 0.15 M NaCl) or angiotensin II (1000 pmol/kg/min in 0.15 M NaCl) for 10 minutes. The results of infusion of both vasoactive compounds showed an initial increase in CBF followed by a slow steady return to baselevel during sustained elevations in systemic blood pressure. To address the question of whether autoregulation of CBF could be induced while systemic blood pressure was maintained at baselevels, vasoactive compounds were infused into the supplying vessels of the cochlea to induce local changes in CBF independent of changes in systemic blood pressure. This was accomplished by inserting a micropipette (external diameter approximately 175 microns and internal diameter approximately 125 microns) into the vertebral vessel thus allowing slow infusions (100 nl/min) of angiotensin II, phenylephrine or vasopressin. The results showed that CBF decreases and are suggestive of local vasoconstriction caused by vascular receptor binding in the supplying vessels of the cochlea.

321.2

SINGLE CHANNEL PROPERTIES OF GOLDFISH (*Carassius auratus*) AUDITORY NEURONS *IN VITRO*. R.L. Davis, E.A. Mroz* and W.F. Sewell. Eaton-Peabody Lab., Massachusetts Eye & Ear Infirmary, Boston, MA 02114.

The goal of this work is to characterize the membrane properties of auditory neurons in culture. Single auditory nerve fibers were microdissected from the saccular nerve of the goldfish without the use of proteolytic enzymes and maintained in culture for periods of up to four weeks at room temperature.

The patch clamp procedure was used to record single channel currents from the growing ends of the neurons as well as from axonal membrane that had been acutely removed from the surrounding myelin. Experiments using the cell-attached configuration of the patch clamp technique have revealed ionic channels with conductances ranging from 16 to 120 pS in solutions that select for K^+, Cl^- or Ba^{++} currents. These channels are being systematically categorized according to voltage dependence, ionic specificity and position along the length of the neuron.

321.3

EFFECT OF CALCIUM CHANNEL BLOCKER D-600 ON NEURAL ACTIVITY IN THE LATERAL LINE OF XENOPUS. Steven L. Guth* and Dennis G. Drescher. Lab. of Bio-otology, Wayne State Univ., Detroit, MI 48201.

Previous investigations in our laboratory demonstrated that spontaneous activity of lateral line nerve fibers of X. laevis is inversely related to the concentration of divalent cation bathing the inner surface of the skin, and that suppression of spontaneous activity by Ca is greater than suppression by Mg (Guth & Drescher, Soc. Neurosci. Abstr. 13: 43, 1987). Our results suggested that transmitter can be released from hair cells in the lateral line without the influx of Ca through voltage sensitive Ca channels (VSCC), and that K-induced depolarization increases neural activity by activation of VSCC. The effect of D-600 on spontaneous activity was studied to test this hypothesis. One uM D-600 (K=2 mM; Ca=0.45, 0.9 or 1.8 mM) had no effect on spontaneous activity. At 5 uM D-600 inhibition increased with Ca concentration. In a paired comparison (n=4) of the influence of 1 mM vs 3 mM K (1.5 mM Ca) on the effect of 2.5 uM D-600, spontaneous activity in 1 mM K was 32% of spontaneous activity in 3 mM K, and D-600 inhibited spontaneous activity 30% in 1 mM K and 60% in 3 mM K. We propose that elevated K increases firing rate by depolarization of the hair cells, which increases Ca current through VSCC, producing a Ca (and D-600) sensitive release of transmitter, while in reduced K (hyperpolarizing) and/or reduced Ca, spontaneous activity is sustained by a mechanism which is less sensitive to D-600. Higher concentrations of D-600 can reduce spontaneous activity further; indeed D-600 is known to inhibit many processes unrelated to VSCC at concentrations greater than 1 uM (Miller & Freedman, Life Sci. 34: 1205-1221, 1984). (Supported by NIH NS16166 to DGD.)

321.4

ELECTROCHEMICAL ASPECTS OF MONOVALENT IONS OF THE STRIA VASCULARIS IN THE CHINCHILLA. K.Ikeda* and T.Morizono* (SPON: M.A.Ruggero). Lab. of Otophysiology, Dept. of Otolaryngology, Univ. of Minnesota Med. Sch., Minneapolis, MN 55414.

The electrochemical profile for K, Na, and Cl ions across the lateral wall of the scala media (SM) was investigated using ion-selective microelectrodes. Healthy chinchillas were anesthetized with ketamine hydrochloride (20 mg/kg). Artificial respiration was maintained and muscular relaxation was induced. After removal of the bony wall of the SM, the double-barreled ion-selective microelectrode successively impaled the spiral ligament (SL), basal cells (BC), intermediate cells or intrastrial space (I), and marginal cells (MC) to reach the SM using an autodrive manipulator at the rate of 1-2 μm/sec. The changes of the membrane potential and ion-selective electrode potential were recorded on the microcomputer at 0.5 sec intervals. The membrane potential from the SL in mV and the K, Na and Cl concentration in mM (mean±S.D.) are respectively as follows: 0 (n=30), 6.6±2.4 (n=10), 146.6±4.4 (n=10), and 109.8±4.6 (n=9) in SL; 4.8±8.5 (n=28), 100.1±16.8 (n=13), 33.9±20.2 (n=5) and 70.2±9.4 (n=6) in BC; 60.4±9.5 (n=32), 22.1±14.9 (n=10), 55.4±21.2 (n=9) and 55.1±14.3 (n=13) in I; 78.3±5.7 (n=27), 141.7±9.1 (n=10), 1.7±1.2 (n=10) and 117.6±21.5 (n=7) in MC; 78.1±4.1 (n=30), 143.0±10.3 (n=10), 1.5±0.7 (n=10), 143.3±13.9 (n=10) in SM. On the basis of the electrochemical potential gradient calculated from the present results, the transport mechanisms of monovalent ions in the stria vascularis were investigated.

(Supported by Grant # 5P50-NS-14538 from the NINCDS.)

321.5

DISCRETE CHARGE MOVEMENTS IN OUTER HAIR CELLS MAY DRIVE THE COCHLEAR FORCE GENERATOR. J.F.Ashmore. Dept. Physiology, Med. Sch., Bristol BS8 1TD, England.

Outer hair cells of the mammalian cochlea are the candidates for cellular generators of active mechanics within the cochlea. The cells generate potential-dependent longitudinal forces vectorially decoupled from forces acting on the apical transducer. The Cell length changes are ATP-independent and operate at acoustic frequencies, (Ashmore J.Physiol 388:323, 1987).

By summing paired pulse responses, as in gating current protocols, small asymmetric currents (<20pA) were measured in outer hair cells isolated for whole cell recording from the guinea pig organ of Corti. Experiments were conducted in bilateral Cs; Ca_i was buffered from the pipette. The currents decayed exponentially and were blocked by F_i. The results can be described by a translocated positive charge, (density about one elementary positive charge per 40nm sq.), equidistributed across the membrane at -30mV. The translocation rate matched *in vitro* rates of length change and suggests that charge movements, possibly associated with the membrane cytoskeleton, are closely linked to the force-producing step.

Supported by the Medical Research Council, U.K.

321.6

HAIR CELL AND PRIMARY AFFERENT RESPONSES IN AN IN VITRO PREPARATION OF THE ALLIGATOR LIZARD COCHLEA. R. A. Eatock. Dept. of Physiology, University of Rochester, Rochester NY 14642.

Understanding of stimulus processing by hair cells has benefited greatly from *in vitro* preparations that provide access to the hair cells' mechanosensitive surfaces. We have extended this kind of preparation to include the primary afferent (cochlear) neurons, using the auditory organ of the alligator lizard as a model system. The organ is excised and maintained at 20-25°C in an artificial perilymph (in mM: 168 or 164 Na^+, 2 K^+, 2 or 4 Ca^{2+}, 3 D-glucose, 5 Hepes). Hair cells are stimulated by moving individual hair bundles with stimulus probes attached to piezoelectric bimorphs (Corey and Hudspeth, J. Neurosci. Meth. 3:183, 1980). Intracellular potentials are recorded from hair cells and distal processes of the primary afferent neurons. As in other preparations, the hair cell responses may be several millivolts, saturate for large stimuli (>1 micrometer), and show frequency tuning. Postsynaptic potentials can be recorded from an afferent fiber in response to stimulation of a single hair bundle. These results suggest that this preparation will be useful for examining stimulus processing by both pre- and post-synaptic elements of the primary afferent synapse.

Supported by NIH and the Deafness Research Foundation.

321.7

REGIONAL DIFFERENCES IN SACCULAR MORPHOLOGY AND EFFERENT RESPONSE IN THE TOADFISH, OPSANUS TAU A. Steinacker and D. N. Menton* Depts. Otolaryngology/ Anatomy Neurobiology, Washington University Sch. of Med., St. Louis, MO. 63110.

As part of a study of the toadfish efferent acousticolateralis system, a morphological and electrophysiological study of regional differences in the saccule was undertaken Scanning electron microscopy was used to define regional characteristics of hair cell stereocilia and kinocilia. Pronounced differences were found in these features in anterior, isthmus and posterior segment of the macula, with the longest kinocilia in rim areas of every segment. Intracellular recordings of spontaneous activity were taken from primary afferents from these 3 regions before and following electrical stimulation of the efferent nucleus in the medulla. Both inhibitory and excitatory effects of efferent activation were found, with inhibition being most prominent in the posterior saccule. Both effects were of prolonged duration following short stimulation pulses. The excitatory response often appeared only after a charateristic delay. This information correlates with the differential regional responses of saccular hair cells to acetylcholine as measured by patch clamp methods.

321.8

"BUNDLE BLOT" PURIFICATION OF HAIR CELL STEREOCILIA: ELECTROPHORETIC PROTEIN MAPPING G.M.G. Shepherd, B.A. Barres, and D.P. Corey. Neuroscience Program, Harvard Medical School; Neuroscience Group, Howard Hughes Medical Institute, and Department of Neurology, Massachusetts General Hospital, Boston MA 02114.

In search of the molecular basis of transduction and adaptation in vestibular hair cells, we have developed a method for the rapid and specific isolation of stereocilia from hair cells of the bullfrog sacculus. The sensory epithelium is stripped of its overlying otolithic membrane and the apical surface gently pressed onto nitrocellulose paper (NCP) with a force of 5-15 millinewtons. The tips of the stereocilia adhere; when the NCP is separated from the sacculus the stereocilia break at their narrow bases and are remain with the NCP. Rhodamine-phalloidin staining and scanning electron microscopy demonstrated that the yield of stereocilia is over 90% while all other cell components are virtually non-adherent.

Using this "bundle blot" method we have studied the electrophoretic pattern of stereociliary proteins. After elution in 1% SDS and 2-mercaptoethanol the proteins were separated in gradient mini-gels and silver stained. Major bands at 42 and 17 kDA co-migrated with actin and calmodulin, respectively. We have previously shown calmodulin immunoreactivity in stereocilia of dissociated hair cells. About 11 other proteins are enriched in stereocilia, at 25, 36, 51, 60, 68 (probably fimbrin), 74, 82, 90, 100, 135, and 220 kDa. A number of proteins present in the cell bodies were not in stereocilia, further indicating the specificity of the isolation. This electrophoretic map differs from that previously reported for intestinal microvilli, probably reflecting the functional differences of these structurally similar organelles. The identity of the stereociliary proteins is under investigation using Western blot analysis.

321.9

OUTER HAIR CELL MOTILITY STUDIED IN AN APPROPRIATE IONIC ENVIRONMENT. B.N. Evans*, R. Hallworth and P. Dallos, Auditory Physiology Lab. & Dept. of Neurobiol. and Physiol., Northwestern University, Evanston, IL 60208.

Several investigators have shown that isolated cochlear outer hair cells (OHCs) exhibit frequency-following motility in response to electrical stimuli. *In vivo*, OHCs exist at the interface of two different ionic media. A high potassium intracellular-like medium (endolymph) bathes the apical face, while a high sodium extracellular-like medium (perilymph) surrounds the basolateral face. In addition, the apical face is electrically polarized by approximately +80 mV. In all previous motility studies, isolated OHCs have been immersed in a high sodium medium only.

Using the approach of Baylor and colleagues (Baylor, Lamb & Yau, J. Physiol., 288, 589, 1979) we have developed a preparation in which OHC motility can be studied in an appropriate ionic and electrical environment. Isolated guinea pig OHCs prepared by conventional methods were gently aspirated into close-fitting fire-polished pipettes containing artificial perilymph. A seal was formed near the apex which was sufficient to separate the electrolytes environments inside and outside the pipette. OHC movements evoked by current applied through the pipette were measured by a photodiode placed over the cell nucleus and all responses were recorded on videotape.

When artificial endolymph was applied outside the pipette, the resting length of the cell in the pipette remained constant for up to 30 minutes. All OHCs outside the pipette rounded up in response to the potassium challenge, as has been reported previously. Under these conditions electrical motility was observable at as large or larger amplitudes than measured with conventional closely-applied pipettes. This demonstrates that OHC motility can be studied under conditions which more closely resemble those found in the intact cochlea. [Supported by NS08635 & NS07223 from NINCDS].

321.10

KAINIC ACID RECEPTOR IDENTIFIED IN THE FROG LABYRINTH USING MONOCLONAL AND POLYCLONAL ANTIBODIES. C.J. Dechesne*, K. Wheaton*, G. Goping*, D.R. Hampson and R.J. Wenthold (SPON: J. Fex) Lab. Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD 20892.

As pointed out by Klinke (Hearing Res.,22:235,1986), the role of glutamate as the neurotransmitter of the synapse between the hair cells and afferent fibers in the cochlea, vestibule and other acoustico-lateralis receptors, remains to be clarified. Identifying the postsynaptic receptors of excitatory amino-acids is an option of choice for their intervention as neurotransmitters. Hampson and Wenthold (JBC,263:2500,1988) have purified a kainic acid receptor from frog brain and produced polyclonal (Wenthold et al.,1987) and monoclonal (Hampson et al., this meeting) antibodies.

Frog (Rana pipiens) vestibules were fixed in situ with 4% paraformaldehyde and .1% glutaraldehyde. Saccules were dissected, processed for cryostat (10μm thick) or vibratome (50μm thick) sectioning and stained with the avidin-biotin method. For E.M., vibratome sections were then processed for epon embedding.

Immunoreactivity with polyclonal antibodies was observed in vestibular afferent fibers from the point they reached the sensory epithelium. Myelinated afferent fibers, vestibular ganglion somata and hair cells were not stained. Electron microscopic observations showed that afferent endings contacting the sensory cells were stained with staining especially heavy at the postsynaptic membranes. Immunoreactivity patterns with a monoclonal antibody were identical to those with the polyclonal antibody, at the light level. These findings suggest that a kainic acid receptor is present at the postsynaptic site of the synapse between hair cells and afferent fibers in the frog.

321.11

TUNING PROPERTIES OF TUBEROUS ELECTRORECEPTORS. E.S. Olson* and L.D. Smullin* (SPON: L. Frishkopf). Depts. of Physics and Elec. Eng., M.I.T., Cambridge, MA 02139.

We measured the frequency dependent impedance of small areas of the electroreceptor/skin structure of two species of Gymnotid electric fish, and used the data to make an equivalent circuit model of the structure. (The qualitative form of the model was proposed by M.V.L. Bennett, in Lateral Line Detectors, P. Cahn, ed., 1967.) The quantitative model allows us to estimate the voltage across the innervated membrane of the electroreceptor cells. The electroreceptor-voltage frequency tuning that we estimate is as sharp as the selectivity that exists neurally at frequencies close to the most sensitive frequency, but the agreement decreases at high frequencies, where the electroreceptor selectivity levels off, while the neural selectivity continues a steep cut-off. Additional low-pass filtering (plausibly in the chemical synapse between the receptor cells and afferent fiber) would reconcile the difference. We show that additional low-pass filtering is also suggested by the phase-versus-frequency behavior of the nerve spike.

Our measurements usually supported the passive linear system model. However, spontaneous electroreceptor voltage oscillations were detected in some measurements, indicating that the electroreceptors sometimes operate in a regime of active nonlinearity. Nonlinear analysis of the Hodgkin-Huxley type equations that we used to describe membrane impedance showed that enough negative damping can lead to sustained oscillations. Near-cancellation of positive and negative damping terms produces strong voltage dependence in the damping at voltages close to the equilibrium voltage, leading to stimulus-level dependent "impedance." When driven with current within a frequency band around the spontaneous oscillation frequency, the oscillations entrained to the drive, suggesting that weak spontaneous oscillations do not degrade sensory reception.

321.12

THE MOUSE BRAINSTEM AUDITORY EVOKED POTENTIAL AS FUNCTIONS OF STRAIN AND AGING. M.W. Church and D.W. Shucard. Wayne State Univ., Detroit, MI and SUNY at Buffalo, NY.

Female mice from C57Bl/6, BDF1 and NZB/W strains were studied in age groups of 3-4 and 9 mo. BAEPs were recorded with from screw electrodes from unanesthetized, restrained animals. The BAEP was comprised of 4 vertex-positive components (labelled P1 through P4). There were significant effects for stimulus intensity (I), mouse strain(S), and the S x I interaction for all amplitude and latency measures. For example, latencies shortened and amplitudes increased with increasing stimulus intensity. Further, the NZB/W strain had the shortest P1 latency, the longest P4 latency, and the largest BAEP amplitudes. The P4-P1 interpeak latency (IPL) also varied significantly as functions of S, I, and the S x I interaction. Here, the BDF1 strain showed a shortening of the IPL with increasing stimulus intensity, while the other two strains showed a lengthening of the IPL between intensities of 50-90 dB, followed by a reverse trend at higher intensities. BAEP thresholds in the 3-4 mo. groups were comparable (Means = 24-31 dB). At 9 months, the NZB/W group showed the least amount of threshold elevation (+6 dB) while the BDF1 and C57Bl/6 strains showed elevations of +17 and +19 dB. These data indicate significant strain-dependent differences in BAEP latencies, amplitudes and age-related hearing loss. Also, the P4-P1 IPL is not constant across stimulus intensities (as some believe) but can vary complexly as functions of stimulus intensity and animal strain.

321.13

AUDITORY EVOKED MAGNETIC FIELDS RECORDED FROM PIGTAIL MONKEY. M. Reite, P. Teale*, J. Whalen*, M. Cox*, and B. Peterson* Dept. of Psychiatry, Univ. of Colo. School of Medicine, Denver, CO 80262.

We recorded the magnetoencephalogram (MEG) over the left hemisphere of a 16 mo old 2.2 kg female pigtail (M. nemestrina) monkey during delivery of 1 kHz 15 ms long tone pips at 75 DB SPL to the right ear. We used a second order gradiometer with 20mm coil diameter, 6 cm baseline, and DC SQUID. Recordings were made from 28 points over a 4x7 grid with 15 mm spacing between points. The subject was sedated with ketamine 10mg/kg and acetylpromazine .55mg/kg. Responses to 128 stimuli were averaged. A 50 ms latency component was identified, and amplitude values obtained with reference to a 200 ms prestimulus baseline. All amplitude values were used to construct spline interpolated mesh plots of the field. The 50 ms latency component exhibited phase reversal on a line extending from approximately the occiput to the bottom of the orbit, the point of phase reversal being approximately over the pinna of the ear. The magnetic field was outgoing anteriorly (positive extrema anterior) and ingoing posteriorly (negative extrema posterior). Both positive and negative extrema were approximately 125 fT in amplitude. Our findings indicate magnetic evoked auditory fields can be recorded from non-human primates, which accordingly may be able to model the human condition. Supported by USPHS MH41396, MH46335, and RR03259.

321.14

EFFECTS OF HEMICHOLINIUM-3 ON BRAINSTEM AUDITORY EVOKED POTENTIAL IN THE RAT. U. Knappe*, F. Wirtz-Brugger*, M. Cornfeldt* and S. Fielding. Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

Acetylcholine (Ach) has been implicated in the brainstem auditory pathway by the use of brainstem auditory potentials (BAEP). (F. Wirtz-Brugger, et. al., Faseb abstracts 1985).

Hemicholinium (HC-3) inhibits Ach synthesis by blocking the high affinity uptake of choline and thereby causes depletion of Ach required for cholinergic transmission (Finberg et. al., Life Sci. 25: 147-156, 1979). The purpose of the study was to provide proof for direct Ach involvement in the generation of the BAEP waveforms. BAEP's were generated in anesthesized rats using bilateral auditory click stimuli and averaged with a Pathfinder I. The following compounds were evaluated in a repeated measurement design: HC-3 @ 60 μg icv significantly decreases BAEP waveforms; Physostigmine (P) @ 0.01 mg/kg ip and carbachol (C) @ 5 μg icv both increased Wave VI significantly. C pretreated with HC-3 reversed the effects of HC-3 and significantly increased Wave VI amplitudes. P did not alter decreases caused by HC-3 suggesting a direct involvement of Ach in the BAEP.

321.15

STIMULUS DEPENDENCIES OF THE GERBIL BRAINSTEM AUDITORY EVOKED RESPONSE (BAER). H.F.Voigt and R.Burkard*. Biomed. Eng. and Com. Dis., Boston Univ., Boston, MA 02215.

Three experiments evaluating the effects of various stimulus manipulations on the click-evoked gerbil BAER are reported. Five replicable peaks (i through v) are observed within six ms of click onset. Click polarity and level were covaried in Exper. 1. There is a parallel increase in peak latencies and a decrease in peak amplitudes with decreasing click level. Exper. 2 covaried click rate and polarity. Peak latencies increased with increasing click repetition rate; this latency increase was greater for the later BAER peaks, producing an increase in the i-v interval. As rate increased, the amplitudes of waves i and v decreased monotonically, whereas the amplitudes of waves ii, iii and iv were largely uninfluenced by click rate. For both experiments, the amplitudes of waves i and v were dependent on click polarity; at high click levels and low rates, wave i was larger to rarefaction clicks and wave v was larger to condensation clicks. Exper. 3 evaluated the effects of click polarity on BAERs to high-intensity, low-rate clicks. In 8 of 10 gerbils evaluated, wave i amplitude was larger to rarefaction clicks, and in all 10 animals wave v was larger to condensation clicks. The effects of click level and rate on BAER peak amplitudes, latencies and interwave intervals are reminiscent of stimulus dependencies reported for the human BAER.

321.16

EFFECTS OF 6-CYANO-2,3-DIHYDROXY-7-NITRO-QUINOXALINE (CNQX/FG9065) ON GUINEA PIG COCHLEAR POTENTIALS. R.P. BOBBIN AND J.-L. PUEL* AND M. FALLON*. Kresge Hearing Research Lab., LSU Medical Center, New Orleans, LA 70112.

Evidence exists to suggest that the hair cell transmitter in octavolateralis systems is an excitatory amino acid such as glutamate. However there is a paucity of excitatory amino acid receptor blockers that are effective in altering the magnitude of the compound action potential of the auditory nerve (CAP). Notably without effect are the NMDA type of excitatory amino acid antagonists, but kynurenic acid, a broad spectrum blocker, is effective (Bobbin, R.P. and Ceasar, G.G., Hear. Res., 25:77, 1987). This study addresses the question of whether a new non-NMDA antagonist, 6-cyano-2, 3-dihydroxy-7-nitro-quinoxaline (CNQX/FG9065; Ferrosan), affects the CAP. Perilymph spaces of guinea pig cochleae were perfused with Ringer solutions containing up to 500 µm concentrations of CNQX at a rate of 2.5 µl/min. for 10 min. Immediately after each period of perfusion cochlear potentials were recorded from a wire inserted in the basal turn scala vestibuli. CNQX as low as 3.3 µm suppressed the CAP. CM and SP were not affected. To date, CNQX is the most potent drug tested which presumably acts at receptors which are involved in the generation of the CAP.

(Supported by an NSF research grant BNS-8419241, Kresge Foundation and the Louisiana Lions Eye Foundation.)

321.17

AGE-RELATED CHANGES IN VERTEX AND TEMPORAL COMPONENTS OF MIDDLE AND LONG LATENCY AUDITORY EVOKED POTENTIALS. A.T. Cacace* and J.R. Wolpaw (SPON: L. Nelson). Depts. of Surgery, Neurology, and Anatomy, Albany Medical College, Albany, NY 12208 and Wadsworth Center for Labs and Research, New York State Dept. of Health, Albany, New York 12201.

Anatomical and physiological changes in the auditory periphery, central pathways, and cortex occur with age. Non-invasive electrophysiological correlates of age-related changes, especially changes in auditory cortex, would enhance understanding of the normal aging process and increase ability to monitor aging and recognize abnormality.

We studied click-evoked AEP scalp topographies (20-256 ms) in 59 subjects (30 males, 29 females, 20-86 years) with no significant history of otological or neurological disease. All had pure tone audiometric evaluations. AEPs were recorded from 32 channels, all referenced to a balanced noncephalic reference. We assessed both temporal components, which are putative products of auditory cortex, and vertex components.

Combined regression analyses for both males and females showed significant age-related changes in the latency of vertex components Pa (.07 ms/yr, p<.01) and N1 (-.14 ms/yr, p<.01), and in the latency of temporal component TP41 (.04 ms/yr, p<.01). TP41 amplitude also changed significantly with age (.02 uV/yr, p=.013), and this change was greater in females than in males.

(Supported by NIH 19891.)

321.18

THE ROLE OF NEURAL ELEMENTS (CELLS AND NERVE FIBERS) IN THE GENERATION OF THE AUDITORY BRAINSTEM POTENTIAL. M. Zaaroor*, A. Starr, Lab of Experimental Neurophysiology, Dept. of Neurology, Univ. of Calif., Irvine, CA 92717

The neural origins of the brainstem auditory evoked potentials are still uncertain. In particular the role of various neural element (cells, fibers) in the auditory brainstem responses (ABR's) has not been decided. In order to provide information to resolve these problems we made lesions in the brainstem auditory pathway of the cat that were relatively selective for destroying cell bodies (Kianic acid) or the myelin of nerve fibers (Lysophosphatial Choline-LPC). Kianic acid (1%, 0.2-0.4 ul) for destroying nerve cells or LPC (10 mg/ml, 0.2-0.4 ul) for destroying myelin was injected to the region of the superior olivary nucleus and the trapezoid body in the brainstem of cats. The ABR's of these cats were studied preoperatively and for up to 45 - 50 days post operatively in a chronic unanesthetized condition. The effects of these selective neuronal and myelin lesions were different than those resulting from electrolytic lesions of these structures. The evidence suggests that both the nerve cells and the fibers contribute to the generation of ABR's components.

321.19

ADAPTATION AND RECOVERY OF AUDITORY NERVE NEUROPHONIC (ANN) RESPONSES. T.C. CHIMENTO*, C.E. SCHREINER, AND R.L. SNYDER*, Coleman/Epstein Laboratory, University of California, San Francisco, CA 94143

Determining the time constants of adaptation and recovery for long duration, low frequency tones in the auditory nerve is an essential part in the understanding of peripheral processing of sustained acoustic signals. ANN responses to 800Hz, 100ms tones presented at 10-30 dBSL have been recorded using bipolar platinum-iridium electrodes placed on the VIIIth N of the cat to determine if the ANN accurately reflects the characteristics of single unit responses.

Adaptation of auditory fiber responses can be fit to the equation $R(t)=(100-\sigma)e^{-t/\tau A}+\sigma$ (Eggermont, 1985 *Hear. Res.*18:57-71) where R(t) is the percent of the response at time t re: onset amplitude, σ is the percent of steady state re: onset amplitude, t is time after stimulus onset and τA is the adaptation time constant. The cat ANN data fits this equation. τA is invariant for each animal at all stimulus levels, with a range of 10-30ms across animals. Recovery of the ANN onset from a 100ms masker fits the equation $R(\Delta t)=(100-\omega)e^{-\Delta t/\tau R}$ (Harris & Dallos, 1979 *J.Neurophys.* 42:1083-1107) where R(Δt) is the percent of the masked response re: unmasked response at time Δt, ω is 100-(no masker response amp/masker response amp at Δt=0), Δt is the delay between masker offset and probe onset, and τR is the recovery time constant. For 300ms probe tones at Δt=0 the recovery of the response as a function of time after probe onset also fits the equation. In both cases τR varys with masker intensity and ranges from 35-125ms.

These results indicate that τA and τR for the ANN are comparable to those measured using single unit techniques and suggests that the ANN can be used to accurately determine the τA and τR of auditory fibers using low frequency, low amplitude, long duration tones. It has become possible to record ANN responses directly from the auditory nerve in patients undergoing surgery for removal of acoustic neuromas and transection of the vestibular nerve to relieve intractable vertigo.The physiological results can be directly related to psychophysical measures determined in the same individual.(Supported by Coleman Fund and NIH Grant NS-16361).

321.20

THE MULTIPLE GENERATORS OF THE AUDITORY MIDDLE LATENCY RESPONSE ***N. Kraus, T. McGee Michael Reese Med Ctr and Univ of Chicago, Chicago, IL 60616**

In the guinea pig and gerbil, auditory evoked middle latency (MLR) components recorded from the midline differ from those recorded over the temporal lobe. These differences are apparent with: intracortical injection of neural inactivating agents (lidocaine and kainic acid), temporal lobe ablation, electrolytic lesions, systemic anesthesia, stimulation rate, and maturation.

Data reveal that midline and temporal lobe MLR components vary independently, suggesting mediation by different generator sources. The particular orientation of the generators responsible for the MLR in the guinea pig and gerbil facilitates the identification of individual components, whereas in humans, MLR component Pa is distributed over widespread areas of the cortical surface, possibly masking the activity of the multiple generators likely to underlie the response. Our data support the existence of multiple MLR generators in laboratory animals and provide insight into the generators of the MLR in humans.

The different course of development observed in midline versus temporal lobe components may help explain why the MLR is inconsistently obtained in children. The lability of the response in humans may occur because one of the generators, presumably the temporal lobe generator, has not yet developed, although other generators may have already matured. (Supported by NIH-NINCDS RO1 NS 21150.)

322.1

MODULATION OF PROTEIN KINASE ACTIVITY IN REGENERATING GOLDFISH OPTIC NERVE. Denis Larrivee, Department of Physiology, Cornell Univ. Med. Coll., New York, NY 10021.

Two-dimensional gel electrophoresis was used to monitor ^{32}P incorporation and abundance of phosphoproteins in goldfish optic nerve during regeneration. Four proteins, including the goldfish equivalent of GAP-43, increased their ^{32}P incorporation in parallel with increases in their abundance. Three proteins showing decreased phosphorylation also showed a parallel decrease in abundance. Thus, the changes in phosphorylation of tnese proteins are likely to be due to changes in their abundance. However, 4 proteins, including a vimentin-like protein, ON2, which decreased their phosphorylation during regeneration, increased in abundance when phosphorylation levels declined. Autoradiography of the separated phosphoproteins also revealed 4 proteins that shifted their isoelectric points during regeneration, including ON2 and a protein resembling the chartin proteins by molecular weight, isoelectric point, and solubility in aqueous buffer containing 1 mM Ca^{2+}. One-dimensional peptide mapping showed that the proteins from normal nerves contained peptides identical to those in the corresponding proteins from regenerating nerves. Acid hydrolysis indicated that ^{32}P was incorporated into phosphoamino acids in all proteins. Thus, changes in phosphorylation of some proteins during regeneration are due to changes in the activity of their protein kinases.

Supported by grants NS-09015 and NS-14967 and a fellowship from SCRF(PVA)

322.2

COMBINED SUBTRACTION/DIFFERENTIAL HYBRIDIZATION SCREEN FOR GENES INDUCED IN OPTIC NERVE REGENERATION. M. E. LaBate* and J. H. P. Skene. Dept. of Neurobiology, Stanford Univ., Stanford, CA 94305-5401.

Direct analysis of axonal proteins by several laboratories has identified a small number of proteins whose synthesis is widely correlated with axon growth. To complement these protein studies, we have used combined subtractive/differential hybridization as a general screen for genes induced during optic nerve regeneration in a large-eyed goldfish, the blackmoor. Poly A^+ RNA was prepared from control retinae (C) and from "regenerating" retinae taken 10 days after optic nerve crush (R). Each of these cDNAs was then hybridized to an excess of control poly A^+ RNA to an R_0t of 800. The cDNA remaining unhybridized in each reaction ("C - C" and "R - C", respectively) was then used to probe duplicate plaque filters of a cDNA library prepared from regenerating retinae. Of 100,000 clones, 90 (0.09%) gave a moderate to strong differential signal with the subtracted "R - C" probe compared to that seen with the subtracted "C - C" probe. Work is now in progress to determine the number of different mRNA sequences represented in this group of clones.

The identity of one mRNA induced during fish optic nerve regeneration was established using a rat GAP-43 cDNA probe . A 1.4 kB fish cDNA selected with the rat GAP-43 probe hybridizes on Northern blots to a 1.4kb mRNA that is strongly induced in fish retina after optic nerve injury. Preliminary sequence analysis indicates that the amino-terminal portion of GAP-43, including a putative membrane-binding domain, are highly conserved between rat and fish.

Supported by NIH grant NS20178 and the Isabelle Niemela Trust.

322.3

RECAPITULATION OF THE DEVELOPMENTAL PATTERN OF GENE EXPRESSION DURING AXONAL REGENERATION: COORDINATED EXPRESSION OF GAP-43 AND A SPECIFIC ISOTYPE OF BETA TUBULIN. **Paul N. Hoffman, Don W. Cleveland* and Mark C. Fishman. Johns Hopkins School of Medicine, Baltimore, MD 21205 (PNH and DWC) and Massachusetts General Hospital, Boston, MA 02114 (MCF).**

Levels of mRNAs for the 68-kD neurofilament protein (NF68), GAP-43, and several isotypes of beta tubulin (classes I, II and IV) were measured in lumbar sensory neurons of rat using hybridization with cloned cDNA probes. During both development and regeneration mRNA levels were relatively low (compared to mature neurons) for NF68 and high for both GAP-43 and the class II isotype of beta tubulin; mRNA levels changed relatively little for the class I and class IV isotypes. The induction of GAP-43 and class II beta tubulin after axotomy followed identical time courses suggesting that the expression of these proteins is closely coordinated. Thus, the longitudinal growth of both developing and regenerating axons correlates with the expression of GAP-43, a protein undergoing rapid axonal transport, and class II beta tubulin, a cytoskeletal protein. NF expression correlates with the radial growth of maturing axons.

322.4

B50/GAP43 IN THE REGENERATING PRIMARY OLFACTORY PATHWAY. J. Verhaagen*, A.B. Oestreicher+, M. Grillo, H. Nielander+, P. Schotman+, W.H. Gispen+, and F.L. Margolis. (SPON: R. Horn). Roche Inst. Mol. Biol., Nutley, N.J. + Inst. for Mol. Biol., Utrecht, Holland.

B50/GAP43 was studied in the primary olfactory pathway following unilateral bulbectomy (UBX) or lesioning of the olfactory epithelium by intranasal irrigation with Triton X-100 (TX). In intact adult animals B50/GAP43 is exclusively present in a subset of differentiating cells adjacent to the basal cell layer of the epithelium. TX-lesioning results in an initial parallel decline of B50/GAP43 and olfactory marker protein (OMP) levels. This reflects the elimination of all mature (OMP positive) and differentiating (B50/GAP43 positive) neurons. The subsequent increase in B50/GAP43 expression during the period of reconstitution of the epithelium and the sequential rise in OMP and decline in B50/GAP43 levels is consistent with the activation of a stem cell population recapitulating normal ontogeny to reconstitute the epithelium. UBX results in ipsilateral increases in B50/GAP43 mRNA and protein. In contrast to the time course following TX (which left the bulb intact) the levels of B50/GAP43 remain elevated up to 75 days after UBX, while OMP expression is minimal. Thus, the different time courses of expression of B50/GAP43 and OMP following TX lesion or UBX demonstrate the role of the olfactory bulb as a target in the reconstitution of the neuroepithelium.

322.5

SOURCE OF GAP43 (B50)-LIKE IMMUNOREACTIVITY (GBLI) IN EXTRA-AXONAL STRUCTURES OF REGENERATING PERIPHERAL NERVE. M.A. Bisby, W. Tetzlaff and H. Zwiers*. Dept. Med. Physiol., Univ. Calgary, Alta., T2N 4N1 Canada.

We previously reported that in sciatic and facial nerves of the rat GBLI was present within newly-regenerated axons as expected, but in regions of nerve containing more mature regenerated axons GBLI was also present in extra-axonal structures, identified as Schwann cell bands of Bungner. Although isolated nerve segments incubated in vitro synthesized a protein which overlapped with GAP43 on 2-D gels, peptide mapping showed that it was not identical to B50: furthermore, it was also synthesized by regions of nerve devoid of GBLI. In situ hybridization with a cDNA probe (Basi et al, 1987) did not show detectable GAP43 mRNA in the immunoreactive nerve segments. On the other hand, pre-embedding EM immunocytochemistry using the PAP technique localized GBLI both within axons, especially to the axolemma, and on the outside of Schwann cells, with the extracellular labelling decreasing with distance from the axons. We conclude that GBLI in extra-axonal structures is not due to local synthesis of GAP43 (B50), but may be due to secretion of this protein from the regenerating axons. The extra-axonal localization of GBLI is consistent with our previous finding that only 30% of the GBLI in regenerating nerve is mobile. (Supported by MRC of Canada and AHFMR).

322.6

AXONAL TRANSPORT OF GAP-43 IN INJURED AND REGENERATING RETINAL GANGLION CELL AXONS OF ADULT RATS. S.K. Doster[1], A.M. Lozano[2], M. Willard[1] and A.J. Aguayo[2]. [1] Dept. Anatomy and Neurobiology, Washington Univ.Med.Sch., St. Louis,Mo., 63110; [2]Neuroscience Unit, Montreal General Hospital and McGill University, Montreal, P.Q., H3G 1A4.

Retinal Ganglion Cells (RGCs) of adult rats increase their immunoreactivity to GAP-43 and can regenerate into peripheral nerve (PN) grafts when their axons are interupted near the eye (Soc.Neurosci.Abst.1987,13:1389). We have now studied levels of axonally transported GAP-43 under similar experimental conditions. ^{35}S-methionine was injected intraocularly in adult Sprague-Dawley rats in which the optic nerve was cut 3 mm behind the eye and left in the orbit or cut at 1 mm and replaced with a 3 cm segment of PN graft to allow axonal regrowth (J. Neurosci. 1987, 7: 2894). Transported proteins were analyzed by two-dimensional fluorography of optic nerve (n=11) and graft samples (n=9). The amount of GAP-43 was estimated by densitometry and related to levels of other transported proteins. In axotomized non-regenerating axons, the transport of GAP-43 increased, peaking in the second week and returning to normal by the fourth week after injury. In contrast, in axons growing along grafts, the enhanced transport of GAP-43 was maintained beyond this period. The results suggest that axonal injury near the RGC soma increases the transport of GAP-43 and that this post-injury enhancement may be prolonged by axonal growth.

322.7

REGULATION OF GAP-43 GENE EXPRESSION DURING AXONAL REGENERATION IN SENSORY NEURONS. G.S. Basi* and J.H. Pate Skene (SPON: T. W. Kraft). Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

GAP-43, an abundant phosphoprotein of growth cone membranes and certain mature pre-synaptic terminals, is expressed in correlation with both regenerative, and developmental axonal outgrowth in a broad evolutionary range of organisms. In the rat, GAP-43 is encoded by a single-copy gene whose expression is regulated primarily at the level of transcription. In order to study how GAP-43 gene expression is regulated during regeneration, we have examined its induction in the sensory neurons of the L4 and L5 dorsal root ganglia (DRG) after unilateral injury of the rat sciatic nerve.

Using a rat GAP-43 cDNA clone (Basi et al., 1987, Cell 49, 785-791) to probe Northern blots of RNA isolated from crush and control DRG, we have observed that GAP-43 expression is induced within 12 -24 h in the cell bodies after an injury. GAP-43 gene expression rapidly rises to a maximum by 3 - 4 d post-injury, and is maintained at this level for at-least 28 d post-injury. Neither the magnitude, nor the timing of this induction is affected by the nature of the injury (crush versus cut), nor by removal of a 12 mm segment of the distal stump. The expression declines to control levels between 28 - 40 d post injury. However, if successful regeneration is prevented by a cut injury, or a cut in conjunction with removal of the distal stump, GAP-43 mRNA does remain elevated for a longer period of time (up to 50 d following nerve transection). Furthermore, the data analyzed thus far indicate that this timing of GAP-43 gene induction is independent of the distance of the crush site from the cell body.

The very early induction of GAP-43 gene activity is consistent with the hypothesis that it is a pre-requisite for, rather than a consequence of, axonal outgrowth. In order to more definitively address this issue we employed colchicine injection into the injury site to prevent axonal outgrowth. Using doses of colchicine which were effective in blocking retrograde transport of a fluorescent tracer, we have observed that injection of colchicine into the nerve does not: a) delay the induction of GAP-43 gene activity by an injury; b) induce GAP-43 gene activity by itself in the absence of a crush. Thus, our observations indicate that GAP-43 expression precedes axonal outgrowth.

This work was supported by NIH grants NS 08096 (GSB) and EY 07397 (JHPS).

322.8

GAP-43 INDUCTION IN REGENERATING DORSAL ROOT GANGLION CELLS: AN ANALYSIS OF SORTING IN AXONAL TRANSPORT. D.J. Schreyer and J.H.P. Skene Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305

In addition to elliciting the regenerative response of the peripheral branch of dorsal root ganglion (DRG) axons, sciatic nerve injury also enhances the propensity of the central branch of DRG axons to regenerate through sciatic nerve grafts within the spinal cord (Richardson, P.M. & Verge, V.M.K., J. Neurocytol. 15:584-594, 1986). It is of interest to determine if a cellular response to injury including the production of axonally transported 'growth-associated proteins' (GAPs) affects the growth propensity of a distant, uninjured axon branch. Thus, we have used Western blot analysis of central and peripheral branches of DRG axons to determine if increased levels of the protein GAP-43 are involved in the enhanced regenerative propensity of central axon branches in response to peripheral injury, or whether newly synthesised GAP-43 is routed exclusively to the injured peripheral axon branch.

Adult rats underwent a unilateral sciatic nerve crush below the sciatic notch and survived 2-8 days. Membrane fractions were prepared from homogenized tissue taken from three areas: the sciatic nerve distal to the L4 and L5 DRG, the L4 and L5 DRG themselves, and the dorsal roots at L4 and L5 proximal to the ganglia. Membrane fractions were solubilized, electrophoresed, electroblotted to nitrocellulose, and probed with a monoclonal antibody specific for GAP-43.

Within 2 days following sciatic nerve crush, GAP-43 immunostaining on Western blots is elevated in comparison to unlesioned control tissue in peripheral sciatic nerve, in DRG, and in central dorsal root segments. The amount of GAP-43 detected in all three areas increases in subsequent days.

The increased amount of GAP-43 synthesized in DRG cells following sciatic nerve crush is thus not exclusively transported to the axon branch sustaining the injury. Transport of induced GAPs to the central branch of DRG axons may therefore be crucial to the enhanced growth ability of central DRG axons that follows peripheral lesions.

322.9

ELEVATED GAP-43 IN SPONTANEOUSLY REGENERATING CNS AXONS OF ADULT RATS. A.P. Foerster, Department of Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5.

Evidence for a spontaneous regeneration of severed axons which formed detours around lesions (made and marked by the implantation of a fine wire cutting device in the brain), that was entirely histological (J. Comp. Neurol. 210:335, 1982), has now been extended by a correlative study with monoclonal antibodies to a neuron-specific, growth-associated protein, GAP-43 (provided by J.H.P. Skene and D.J. Schreyer) and to neurofilaments (210 kDa). (Pentobarbital anesthesia was used.) Immediately postlesion, neither an axonal detour nor elevated GAP-43 was observed. Axonal detours then increased progressively as the number of severed axons facing the lesion was reduced. Within a few days, GAP-43 was elevated in the terminal portions of severed axons, and was observable for at least 3 weeks in those pursuing a reoriented course around the lesions. Within the population of axons now curving around the lesion, GAP-43 was elevated primarily in those closest to its end, i.e. the most recent arrivals, suggesting that it increases early in the regeneration and falls thereafter. There was no evidence (swelling, varicosities) in the detouring axons of mechanical hindrance of axoplasmic transport.

These immunocytochemical findings support the anatomical evidence for the occurrence of spontaneous axonal regeneration after lesions of in the adult rat brain.

REGENERATION: OTHER GROWTH-ASSOCIATED PROTEINS

323.1

PERIPHERAL NERVE INJURY AND REGENERATION INDUCES MODIFICATIONS OF SCHWANN CELL-ASSOCIATED MOLECULES Neuberger, T.J. and Cornbrooks, C.J., Dept. of Anat. and Neurobiol., Univ. of Vt., Burlington, Vt., 05405.

After nerve transection, Schwann cells (Sc) within the distal nerve stump undergo a series of poorly characterized events which may influence neuronal regeneration. Using immunohistochemical methods, we examined the expression of known, Sc-synthesized antigens which are localized in the cytoplasm or on the membrane. The immunoreactivity of glial fibrillary acidic protein (GFAP), a cytoskeletal component specific in ensheathing Sc, diminished after injury, while that of vimentin (VIM), a cytoskeletal component in myelinating Sc, became prominent within most Sc. Upon reinnervation, GFAP-positive filaments increased in number and length within finite domains of the nerve; VIM concomitantly decreased in these same areas. In control and degenerating nerves, C4 (a cell surface protein expressed by Sc in contact with nonmyelinating Sc) closely duplicated the expression of GFAP. However, upon reinnervation, increased C4 immunoreactivity preceeded that of GFAP and was transiently associated with an undetermined component of the Sc cytoskeleton. S-100 protein, normally localized near the inner and outer membrane of myelinating Sc, was slightly diminished after axotomy, but gradually increased during reinnervation. These studies indicate that alterations in Sc-neuron contact result in modifications in the expression of Sc proteins which can in turn can influence Sc shape and function. Supported by PHS RO1 20189.

323.2

ISOLATION AND CHARACTERIZATION OF A FACTOR WHICH INHIBITS PROTEIN MODIFICATION BY LYSINE DURING NERVE REGENERATION. M. Yu*, G. Chakraborty*, S. Shyne-Athwal and N.A. Ingoglia, Dep't of Physiology, New Jersey Medical School, Newark, N. J. 07103

The posttranslational modification of proteins by amino acid addition has been demonstrated in a variety of biological systems. In our laboratory, we have found that these reactions take place in rat sciatic nerves, are greatly magnified two hours after a crush injury and that the addition of individual amino acids appears to be regulated by specific factors (Shyne-Athwal et al., 1986, Science, 231: 603-605). In the present study, we have begun to characterize the inhibitor to posttranslational lysine addition at two hours after crush injury to rat sciatic nerves.

In the first series of experiments, the inhibitor was shown to be heat stable (90°C, 5 min.), and sensitive to treatment with trypsin (0.1% trypsin caused a 50% reduction in inhibitory activity). These findings suggest that the inhibitory factor is a heat stable protein or peptide. When the inhibitor was passed through a Sephacryl S-300 column, inhibitory activity eluted with molecular weight standards of approximately 10-20k Daltons. However, following further purification on a PROTEIN PAK 125 HPLC size exclusion column, the inhibitory factor appeared to have a molecular weight of less than 4,000.

In summary, the inhibitor of posttranslational conjugation of lysine to proteins appears to be a small molecule, which is heat stable and trypsin sensitive. Further characterization of this molecule is in progress. (Supported by grants from the NIH).

323.3

EARLY AND LATE NEUROFILAMENT (NF) PHOSPHORYLATIONS IN DEVELOPMENT AND REGENERATION. D. Dahl. Harvard Medical School and VA Medical Center, West Roxbury, MA. 02132.

We previously reported on the late occurrence of NF phosphorylation in development and regeneration. Here we show that NF phosphorylation is a complex phenomenon and that individual NF phosphorylation events either occur shortly after NF expression or following a considerable delay. NF phosphorylation was studied by indirect immunofluorescence in tissue sections and in primary dissociated cultures of spinal cord and dorsal root ganglia with 16 monoclonal antibodies reacting with phosphorylated epitopes. The antibodies either decorated NFs shortly after their appearance (as indicated by double labeling experiments with NF polyclonal antibodies) or after a considerable delay, ranging from 4 to 9 days in embryonal development, from 6 to 15 days in sciatic nerve regeneration and from 12 to 27 days in primary cultures. With most (but not all) antibodies, there was a good correlation between in vivo and in vitro findings as to the early or late appearance of phosphorylated epitopes. One monoclonal antibody stained regenerated axons one month after transection with an abnormal pattern, thus suggesting differences between normal and regenerated nerves with respect to NF phosphorylation. Supported by the Veterans Administration.

323.4

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE SYNAPTIC VESICLE PROTEIN SV2 IN CULTURED ADULT RETINAL NEURONS. J.W. Mandell*, E. Townes-Anderson, and P.R. MacLeish, Cornell Univ. Med. Coll. and Rockefeller Univ., New York, NY 10021

We have previously shown that mature neurons from the tiger salamander retina can be maintained *in vitro* where they regenerate processes and reform synaptic connections. To visualize the distribution of synaptic vesicles during neurite regeneration, a monoclonal antibody to the synaptic vesicle protein SV2 (Buckley and Kelly, J. Cell Biol., 100:1284 1985) was used in combination with indirect immunofluorescence. Cells were isolated by enzymatic digestion and mechanical dissociation. Rod, cone, and bipolar cells examined immediately after plating had light perinuclear fluorescence and intense staining in synaptic terminals, consistent with vesicle accumulations observed by electron microscopy. Multipolar neurons, which include horizontal, amacrine, and ganglion cells, retained only the proximal portion of their processes and showed only dim perinuclear staining. Intense staining remained in photoreceptor and bipolar terminals for at least 2 weeks; retention of SV2 staining did not depend on the development of cell contacts. In addition, process outgrowth from photoreceptor somas and bipolar axons was brightly immunoreactive. In contrast, new processes from ganglion cells, identified by retrograde labeling, stained only slightly. However, a small percentage of unidentified multipolar cells showed punctate staining in processes. Thus, retinal neurons isolated with axons retain SV2 staining in synaptic terminals and appear to regenerate SV2-rich processes. These findings suggest that synaptic vesicle accumulations in adult neurons can be maintained in the absence of postsynaptic cell contact.

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323.5

CHANGES IN SPECIFIC FAST AXONALLY TRANSPORTED PROTEINS IN CRUSHED FROG AND RAT OPTIC NERVES. G-S. Perng* and G.W. Perry. Department of Physiology & Biophysics, University of Miami School of Medicine, Miami, Florida.

We have seen that the majority, but not all, of those proteins rapidly transported along frog sciatic nerve axons are also conveyed at the fast rate along frog optic nerve fibres, and that a similar pattern also exists in rat optic nerve axons. Following crush of the frog optic nerve the labelling of many of the fast transported proteins (FTPs) increases. Most notable among these changes was the large increase in labelling of a GAP-43 like protein in the regenerating frog optic nerve. Also, the labelling of another FTP, designated A1 and which is normally undetectable, increases considerably in the regenerating frog optic nerve. Shortly after crush of the rats optic nerve there is a large increase in the labelling of a transported protein of similar molecular weight and isoelectric point to GAP-43. A very interesting similarity between crushed frog optic and sciatic nerves was the appearance of a polypeptide, designated A25, seen previously to be generated specifically at the site of damage in the sciatic nerve. The precursor to A25 is most likely a fast axonally transported protein, possibly a high molecular weight precursor, designated A30, which was also present in the normal optic nerve. However, A30 but not A25 was also seen in the patterns of FTPs delivered to the terminals of the optic nerve axons, that is in the frogs optic tectum. This suggests that A25 is not present in normal optic nerve or its terminals, and its appearance in the nerve is damage specific. A polypeptide with similar molecular weight and isoelectric point to A25 was also seen in the rats optic nerve shortly after crush. The high molecular weight protein, A30, also appears to be rapidly transported in normal undamaged rat optic nerves.

This work was supported by NIH grant EY06449; G-SP is a Markey Fellow.

323.6

LEVELS OF β-PREPROTACHYKININ (β-PPT) mRNA AND TACHYKININS CHANGE DIFFERENTIALLY IN RAT DORSAL ROOT GANGLIA (DRG) FOLLOWING SCIATIC NERVE SECTION. D.B. Henken[1], A. Tessler[1,2], M.-F. Chesselet[1] and M. Murray[1], [1]The Medical College of Pennsylvania, and the [2]VA Medical Center, Philadelphia, PA.

Localization of a peptide and the mRNA for that peptide within specific populations of DRG neurons was used to examine metabolic changes associated with axotomy and regeneration. In DRG, 15-20% of the total neuronal population contains tachykinins and the mRNA for the tachykinin precursor, β-PPT. It is known that sciatic nerve section at first reduces levels of tachykinins in DRG which later recover when regeneration is complete. We examined levels of tachykinins and β-PPT mRNA 2 weeks and 6 months following sciatic nerve section and re-apposition in order to determine whether the altered peptide production by DRG cells is regulated at the level of gene expression. mRNA containing neurons were visualized with a ^{35}S-labelled RNA probe for β-PPT and tachykinins were demonstrated immunocytochemically. Two weeks following axotomy the proportion of cells labelled for the mRNA or peptide decreased. Eight to 10% of the total neuronal population stained for the peptide, whereas only 3% labelled for β-PPT mRNA. By 6 months post-operatively, the proportion of cells labelled for both the message and the peptide returned to control levels of 15-20%. These results suggest that regulation of metabolic changes during regeneration depends at least in part on alterations in gene expression and that in situ hybridization histochemistry can be used to study this regulation. Supported by NSF grant BNS8616841, VA Medical Research Service, NIH grant NS24707 and USAMRDC grant 51930002.

323.7

REGENERATING AXONS INCORPORATE NEWLY-SYNTHESIZED CYTOSKELETAL PROTEINS. B.A. Reynolds* and M.A. Bisby. (Spon: K.E. Cooper). Dept. Med. Physiol., Univ. Calgary, Alta., T2N 4N1

The structural hypothesis of slow axonal transport (SAT) suggests that regenerating axons derive their cytoskeletal proteins from those pre-formed in the axon, and moving along it at < 4 mm/day. However, the synthesis of some of these proteins in the cell body increases within a few hours after axonal injury, which seems unnecessary if regeneration is sustained by pre-formed proteins.

The L5 DRG of anesthetized adult rats was labeled with 35S-methionine 3 days after crushing the sciatic nerve 60 mm from the DRG. Labelled proteins present in the nerve distal to the crush were analyzed two days later: these included actin and tubulin identified immunologically and by MW and pI. These cytoskeletal proteins synthesized in the cell body 3 days after injury are transported into the regenerating axons, over 60 mm distal from the cell body, at a velocity of at least 30 mm/day.

Our results demonstrate that regenerating axons do incorporate cytoskeletal proteins produced by the cell body post-injury and raise the possibility that these may be special variants of the normal cytoskeletal proteins, which are required for axonal outgrowth. (Supported by MRC and AHFMR).

323.8

CALCIUM ACTIVATED NEUTRAL PROTEASE (uCANP) ACTIVITY IN AXONS IS INCREASED PROXIMAL TO A NERVE CRUSH. D.J. Fink and M. Mata, Neurology Research Laboratory, University of Michigan and VA Medical Center, Ann Arbor, MI 48105.

CANPs are cysteine endopeptidases present in the cytosol which require calcium ions for activity. A CANP activated by millimolar Ca^{++} has been identified in peripheral nerve and degrades neurofilaments in Wallerian degeneration. uCANP, activated by micromolar Ca^{++} has also been identified in peripheral nerve, although its role is not known. We measured uCANP activity in normal nerve and in proximal axons 1 wk after distal nerve crush.

Neurofilaments (NF) were isolated using a modification of the method of Schlaepfer and the CANP activity in the supernatant determined by degradation of ^{14}C casein in the presence of 50 uM Ca^{++}. The enzyme has an apparent Vmax of 70 ug casein/mg crude CANP/30 min and a K_m of 25uM. The activity is linear for 30 minutes, and using our standard incubation conditions varies directly with the amount of CANP.

We found activity in normal nerve was 41.1 ug casein/mug CANP/30 min. The activity of crude CANP isolated from proximal sciatic nerve 1 week after distal nerve crush was 71.1 mg casein/ug CANP/30 min ($P<.05$). Incubation of tritium labeled NF with 10ug of CANP for periods ranging from 1-24 hrs confirmed that uCANP was active in degrading NF, and that the uCANP from the proximal nerve after crush degraded the NF more rapidly than the normal uCANP.

The presence of uCANP in normal nerve suggests that turnover of NF may occur within the axon during axonal transport, and agrees with the presence of NF immunoreactive breakdown products in normal axons. The increased activity of uCANP in proximal nerves after crush may be related to the changes in NF transport which occur after nerve crush.

Supported by VA Merit Review Grants to Dr. Mata and Dr. Fink.

323.9

DIFFERENTIAL EXPRESSION OF 5'-NUCLEOTIDASE ENZYME AND IMMUNOREACTIVITY IN RESTING AND REACTIVE SCHWANN CELLS OF THE RAT SUPERIOR CERVICAL GANGLION. W. Nacimiento*, M.B. Graeber* and G.W.Kreutzberg (SPON: J. Noth). Dept. of Neurology and Clinical Neurophysiology, Alfried-Krupp-Hospital, 4300 Essen, and Dept. of Neuromorphology, MPI for Psychiatry, 8033 Martinsried, F.R.G.

The cellular and subcellular distributions of the ectoenzyme 5'-nucleotidase (E.C.3.1.3.5.) were studied in Schwann cells (SC) of normal and regenerating rat superior cervical ganglia (SCG). 1. 5'-nucleotidase enzymatic activity was absent from SC of normal SCG whereas during regeneration SC exhibited strong enzymatic activity in their perikaryal plasma membranes. 2. 5'-nucleotidase immunoreactivity was present in both normal and axotomized SCG. 3. At the ultrastructural level 5'-nucleotidase immunoreactivity was confined to SC plasma membranes underlying the basal lamina. In conclusion, 5'-nucleotidase enzymatic activity is newly expressed by SC stimulated by postganglionic axotomy and may thus serve as a marker for SC activation during sympathetic nerve cell repair. The differences observed in the subcellular distributions of 5'-nucleotidase enzymatic and immunoreactivity of resting and reactive SC provide another example for the heterogeneity of glial plasma membranes and suggest that the latter is responsive and can be influenced by neuronal injury.

323.10

PLASMINOGEN ACTIVATOR ACTIVITY IS EXPRESSED IN THE REGENERATING GOLDFISH VISUAL PATHWAY. F.J. Salles*, S. Strickland* and N. Schechter (SPON: W. Quitschke). Depts. of Biochemistry, Pharmacology and Psychiatry, SUNY at Stony Brook, NY 11794

In contrast to higher vertebrates, the CNS of lower vertebrates has the capacity for functional nerve regeneration after injury. A useful model to study this phenomenon is the goldfish visual pathway which regenerates following optic nerve crush.

We have studied the possible role in optic nerve regeneration of the protease plasminogen activator (PA), which has been previously implicated in nerve growth. After unilateral optic nerve crush, PA activity appeared in crude homogenates of the crushed nerve. No activity was observed in the contralateral uninjured nerve, nor in sham operated controls. The PA activity was seen as early as one day post-crush, reaching a peak at about 10 days; by 80 days post-crush, at which time vision is restored, the activity is no longer detected. Electrophoretic zymography for PA activity revealed the presence of a major species at 75Kd with two other species of variable intensities at 65 and 36Kd. The PA activity could be partially inhibited with polyclonal antisera against either human tissue-type PA or human urokinase-type PA.

These results demonstrate that PA is present in the goldfish and its expression is correlated with the process of optic nerve regeneration. (Supported by grants EY05212 (NIH) to NS and HD17875 (NIH), BC525H (ACS) to SS.)

323.11

EXTRACELLULAR GLYCOPROTEINS OF THE GOLDFISH OPTIC TECTUM ARE LABELLED BY INTRAOCULAR INJECTION OF ^{3}H-PROLINE. Finnbogi Thormodsson, Edna Antonian* and Bernice Grafstein. Physiology Dept., Cornell U. Med. Coll., New York, NY 10021.

Two-dimensional polyacrylamide gels of goldfish brain showed a prominent group of soluble proteins, consisting of 2 main sub-groups with MW's about 33K and 38K respectively, each containing a number of isoforms with pI 5.0-5.6. The proteins were also present in a small amount in the optic nerve but not in the retina. They could be readily extracted from the tissue by soaking in isotonic medium, indicating that they are largely extracellular. Since they are glycoproteins (as indicated by ^{3}H-glucosamine labelling), we have designated them "exoglycoproteins" (XGP's). They are identical to the "ependymins" described by Shashoua (Cell. Molec. Neurobiol. 5: 183, 1985). The XGP's in the optic tectum became labelled after intraocular injection of ^{3}H-proline. The labelling was consistently higher in the tectal lobe contralateral to the injected eye, it increased during optic nerve regeneration, and it was abolished by intraocular injection of a protein synthesis inhibitor, suggesting that the XGP's might be synthesized in the retina and axonally transported to the tectum. Nevertheless, the XGP labelling was inhibited by intracranial block of protein synthesis. Antibodies to the XGP's produced prominent immunostaining in the pia, whereas neuronal structures were negative. Thus the XGP's can be synthesized in non-neuronal cells of the tectum from precursors derived from axonally transported material. [Supported by NS-09015.]

323.12

IMMUNOCYTOCHEMICAL LOCALIZATION OF A REGENERATION-ASSOCIATED PROTEIN IN THE GOLDFISH VISUAL SYSTEM. G. R. Wilmot*, T. S. Ford-Holevinski, P. A. Raymond, and B. W. Agranoff. Neuroscience Lab and Dept. of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48104-1687.

Retinal proteins that are synthesized in greater amounts following axotomy of the goldfish optic nerve can serve as biochemical probes for understanding the molecular mechanisms underlying CNS regeneration. Such proteins can be radiolabeled and then identified in gels of regenerating optic nerve, indicating that they originate in retinal ganglion cells (RGC's) and also that they are axonally transported. We are studying an acidic 68/70 kDa axonally transported doublet in which the incorporation of ^{35}S-methionine is increased 4-35 days after nerve crush. Here we report immunocytochemical evidence that this cytosolic doublet is indeed localized within RGC's. The 68/70 kDa doublet was purified by DEAE and lectin chromatography of a high speed supernatant of goldfish brain. Following SDS-PAGE, the proteins were electroblotted onto nitrocellulose, and strips containing the doublet were implanted subcutaneously into rabbits. Reactive antiserum, judged by Western blots, was incubated overnight (diluted 1:1000) with aldehyde-fixed cryostat sections of control and 10-15 day postcrush retinas. Bound antibody was visualized by immunofluorescence (goat antirabbit conjugated to FITC) or immunohistochemistry (avidin-biotin-peroxidase conjugate). While immunoreactivity was localized to the RGC's of both control and postcrush retinas, it was markedly increased as a result of axotomy. Pre-immune serum was non-reactive. These results indicate that the 68/70 kDa doublet is synthesized and is present in increased chemical amounts in the regenerating RGC's, and thus serves as a biochemical correlate of functional recovery in the teleost CNS. (Supported by NEI grants EY 05947 and EY 04318.)

323.13

Analysis of the induction of the genes of two putative calcium binding proteins during sciatic nerve regeneration. M. De León, P. Masiakowski and E. M. Shooter. Department of Neurobiology, Stanford University Sch. of Med., Stanford, Ca 94305.

Nerve growth factor is a polypeptide neurotrophic agent that is important in the maintenance and survival of sympathetic and some sensory neurons in the peripheral nervous system. NGF may also be an important element in the series of events that lead to axon growth, and consequently, nerve regeneration. NGF may participate in the regulation of axon growth by regulating the expression of specific genes, the products of which may be important for the growth process. In order to test this hypothesis we have been studying the expression during regeneration, of two mRNAs that have been found to be induced in PC12 cells after NGF exposure. The predicted amino acid sequence of these cDNA clones correspond to the family of S100 calcium binding proteins (Masiakowski and Shooter, P.N.A.S.85:1277,1988). Sciatic nerves from adult rats were cut and allowed to regenerate for 1 to 3 days; the contralateral nerve served as control. RNA was extracted from nerve distal to the injury (or from a similar area in the contralateral nerve), and from the 4th and 5th dorsal root ganglia from both the contralateral and axotomized nerves. The RNA was separated on an 1.2 % agarose formaldehyde gel and transferred to Hybond nylon filters. The filters were probed with ^{32}P-labelled inserts. It was found that both species of RNA were present in the normal nerve and DRG. The steady state RNA levels of both 42A and 42C species are increased during sciatic nerve regeneration. The induction was observed during the first three days of regeneration in the segment distal to the injury.

323.14

CHANGES IN GENE EXPRESSION FOLLOWING AXOTOMY ARE SIMILAR IN RUBROSPINAL (CNS) AND FACIAL (PNS) NEURONS. W. Tetzlaff* and M.A. Bisby. (Spon: W.K. Stell). Dept. Med. Physiol., Univ. Calgary, Alta., T2N 4N1 Canada.

Levels of mRNAs for tubulin (Tm), actin (Am), and GAP43 increase in rat facial motoneurons after facial nerve injury, while mRNAs for neurofilament proteins (Nm) decrease. Rubrospinal neurons react to axotomy not with regeneration, but with atrophy: what effect does axotomy have on expression of these mRNAs?

2 weeks after a C3 hemisection, a-Tm levels in rubrospinal neurons were elevated, but this was no longer evident at 3 weeks. No Am increase was detectable at 2 or 3 weeks, but surprisingly some neurons showed increased GAP43m expression. As in facial motoneurons, a profound decrease in Nm occurred. Even though their axons do not regenerate, rubrospinal neurons react to axotomy with many of the same changes in gene expression as occur in facial motoneurons. We are following the time-course of these changes in more detail to determine if failure of regeneration is associated with inability to sustain them. (Supported by MRC of Canada and AHFMR).

323.15

ALTERATIONS IN CYTOSKELETAL PROTEIN mRNA LEVELS IN REGENERATING MOTOR NEURONS. N.A. Muma, P.N. Hoffman, M.D. Applegate*, C.A. Fleischman*, H.F. Hough*, and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182.

Axonal injury produces characteristic changes in cellular morphology, protein expression, and RNA metabolism. The role of the regulation of genes in regenerative processes can be explored by examining levels of specific mRNA species at various stages following nerve injury/regeneration. To this end, in situ hybridization was used to measure levels of cytoskeletal protein mRNAs in motor neurons of the L4-L5 spinal cord at several intervals following axotomy of sciatic nerve. Fourteen days postaxotomy, levels of the low molecular-weight neurofilament subunit mRNA were maximally reduced (3.5 fold). Similar changes were seen in levels of mRNA coding for other neurofilament subunits. In contrast, levels of β-tubulin mRNA in neurons were increased maximally (twofold) 14 days postaxotomy. Patterns of changes in levels of cytoskeletal protein mRNAs that result from axotomy in these spinal motor neurons of the central nervous system parallel those recognized following axotomy of sensory neurons in the peripheral nervous system. These studies in animal models of regeneration will lay the foundation for studies of cytoskeletal protein gene expression in animal models of degeneration and human neurodegenerative diseases.

323.16

A COMPARISON OF PERIPHERAL AND CENTRAL AXOTOMY ON NEUROFILAMENT AND TUBULIN GENE EXPRESSION IN RAT DORSAL ROOT GANGLION (DRG) NEURONS. J.Wong* and M.M. Oblinger (SPON: C.M. Combs) Dept. Cell Biology and Anatomy, Chicago Medical School, North Chicago, IL 60064.

The cell body of the pseudounipolar dorsal root ganglion (DRG) neuron serves as the synthetic center for both a centrally and a peripherally directed axon. The two axonal branches of DRG cells are known to differ structurally, biochemically and functionally and many previous studies have indicated that aspects of the DRG cell response to peripheral branch injury differ from those elicited by central branch axotomy. In the present study, we asked whether central branch axotomy elicits a similar or different change in cytoskeletal gene expression as does peripheral branch axotomy in adult rat DRG neurons. Unilateral crush lesions of either the L5 dorsal root (4-6 mm from the L5 DRG) or the sciatic nerve (at 50-55 mm from the L5 DRG) were made and the axotomized L5 ganglia and their contralateral controls were harvested between 1d and 8 weeks later. The DRGs were fixed in paraformaldehyde, embedded in paraffin, sectioned at 10 μm, hybridized with ^{35}S-labeled cDNA probes to the mRNAs of NF68 (provided by Dr. N. Cowan, NYU) and beta tubulin (provided by Dr. S. Farmer, Boston U) and subjected to autoradiography. Quantitative analysis of *in situ* hybridization experiments on DRG neurons that sustained a peripheral axotomy revealed that the NF68 mRNA levels were substantially reduced by 1 week after injury. The decrease was maximal 2 weeks after injury and, by 4 and 8 weeks, the NF68 mRNA levels were not significantly different from those in contralateral (control) neurons. In contrast, tubulin mRNA levels were substantially increased (over 200% of contralateral controls) at 1 and 2 weeks after peripheral branch injury and then returned to normal by 4 weeks. At one week after central branch axotomy, the mRNA levels for NF68 and tubulin in DRG neurons were not significantly different from contralateral control values. However, at 2 weeks after central axotomy, NF68 mRNA levels were reduced and tubulin mRNA levels were increased relative to contralateral controls. The overall magnitude of change in cytoskeletal protein mRNA levels was lower after central branch than after peripheral branch axotomy. We conclude that both peripheral and central branch axotomy results in a change in mRNA levels for NF proteins and tubulin. However, the response to central branch injury is of smaller magnitude and has a different time course than the response to peripheral branch injury.

MONOAMINES AND BEHAVIOR IV

324.1

NEUROCHEMICAL BASES OF SPARED INGESTIVE BEHAVIOR IN RATS DEPLETED OF DOPAMINE AS NEONATES.
B.M. Potter* and J.P. Bruno, Dept. of Psychology, The Ohio State University, Columbus, Ohio 43210.

Rats incurring near-total depletions of forebrain dopamine (DA) during development do not exhibit the ingestive and sensorimotor deficits seen when comparable depletions occur during adulthood. However, as with animals depleted as adults, the feeding behavior of rats depleted as infants is disrupted by doses of α-methyl-tyrosine (AMT), an inhibitor of catecholamine (CA) biosynthesis, that have no effect in control animals. We have begun to investigate the mechanism of this AMT effect in adult rats treated with 6-HDA at 3 days of age (Day 3).

The supersensitivity to AMT is probably not due to the drug's effect on peripheral CAs because 6-HDA (sc)-induced sympathectomy prior to AMT did not potentiate its effectiveness. Central norepinephrine (NE) is also not likely to be involved because rats depleted of only NE on Day 3 are not supersensitive to AMT and because rats depleted of both DA and NE on Day 3 are no more sensitive than rats depleted of DA alone. The role of residual DA neurons is unclear. While our previous data suggest that DA is no longer involved in ingestive behavior, we report that administration of haloperidol potentiated the effects of low doses of AMT in adults depleted of DA as infants. We are currently studying the basis of this paradoxical finding.

324.2

SELECTIVE PCP AND SIGMA RECEPTOR AGONISTS PRODUCE DIFFERENTIAL EFFECTS IN RATS WITH UNILATERAL LESIONS IN THE SUBSTANTIA NIGRA. D.J. Hepler, S.W. Tam and V.J. DeNoble. E. I. duPont Co., Med. Prod. Dept., Wilmington, DE 19898.

Phencyclidine (PCP) has been shown to induce ipsilateral turning in rats with unilateral lesions of the substantia nigra. Because PCP is a mixed sigma and PCP receptor agonist, it is unclear which mechanism is involved in modulating the observed turning behavior. In this study, rats with unilateral 6-hydroxydopamine-induced lesions in the substantia nigra were evaluated for changes in rotational behavior following administration of PCP, (+)-SKF 10,047 (N-allylnormetazocine), (+)-pentazocine, MK-801, amphetamine, and apomorphine. The rotation model provides a quantitative means for examining the effects of these compounds on pre- vs. post-synaptic dopamine (DA) neuronal activities. The mixed PCP and sigma receptor agonists PCP (0.1-4 mg/kg s.c.) and (+)-SKF 10,047 (1-20 mg/kg s.c.) produced dose-dependent ipsilateral turning, indicating a pre-synaptic DA effect. This behavior is similar to that produced by amphetamine. In addition, the highly selective PCP receptor agonist, MK-801 also produced strong dose-dependent ipsilateral turning. The potency is MK-801 > amphetamine > PCP > (+)-SKF 10,047. In contrast, apomorphine, a DA receptor agonist, produced contralateral turning behavior. The highly selective sigma receptor agonist (+)-pentazocine (3-60 mg/kg s.c.) did not produce rotation. These results suggest that PCP receptor activation produces pre-synaptic DA release.

324.3

THE SELECTIVE D1 ANTAGONIST SCH-23390 PRODUCES CATALEPSY IN YOUNG RATS. L.W. FITZGERALD* and J.H. HANNIGAN. Center for Behavioral Teratology, SUNY-Albany, Albany, NY 12222.

We assessed the cataleptogenic effects of the selective D1 receptor antagonist SCH-23390 maleate (SCH) in 13-, 17-, and 21-day-old male rat pups. Pups received either SCH (0.25,0.5, 1.0 mg/kg, s.c.) or saline vehicle and were placed in a holding chamber. The chamber for 13- and 17-day-old pups was heated to 32°C in order to simulate nest temperature. Catalepsy, defined as the latency to remove the forepaws from a raised horizontal bar, was assessed at 15,30, and 45 min post-injection. SCH produced monotonic increases in catalepsy in all of the ages tested, though the magnitude of the responses depended on age. Overall, 13-day-old pups were less responsive to SCH than 17- or 21-day-old rats, which responded equivalently to the drug. These behavioral effects coincide temporally with the ontogeny of D1 receptors which peaks to adult levels at 21 days of age (Zeng, W. et al, J. Neurochem.,50:3,1988).This study demonstrates the potent cataleptogenic properties of SCH in the preweanling rat, and may provide a significant functional correlate to the development of D1 receptors.

324.4

ROTATION INDUCED BY SELECTIVE DOPAMINE AGONISTS FOLLOWING ELECTROLYTIC SUBSTANTIA NIGRA (ESN) LESIONS. K.E. Asin, L.Bednarz and W.E.Montana, Neurosci Res Div, Pharmaceutical Discovery, D47U, Abbott Labs, Abbott Park, IL 60064.

Although selective D1 and D2 dopamine agonists produce locomotion in reserpinized (RES) rats and contralateral (CONTRA) rotation in rats with unilateral 6OHDA lesions, D1 agonists do not elicit rotation in rats with quinolinic acid striatal lesions or diencephalic hemitransections. In this study we examined the effects of D1 and D2 agonists in ESN lesioned rats before and after chronic reserpine (RES) treatment.

Rats were prepared with unilateral ESN lesions and, 2 weeks later, were tested for apomorphine-induced rotation. Those showing >90 ipsilateral (IPSI) rotations/60min were later tested in response to SKF38393 (SKF)(0-20mg/kg) or LY171555 (LY)(0-.45mg/kg). LY, but not SKF, produced dose-dependent IPSI rotation, which was blocked by SCH23390 (.01mg/kg). In another group, rats were tested with either SKF (20mg/kg) or LY (.15mg/kg) before and after chronic RES (1mg/kg over 5d). RES potentiated the IPSI rotational response to LY, and resulted in the appearance of strong CONTRA rotation in response to SKF, suggesting different output pathways for the two rotational responses. The IPSI rotation produced by LY was potentiated by SKF coadministration in both control and RES rats. These results suggest that the dopamine receptors interact, in a manner dependent on relative D1:D2 stimulation, to determine the direction of rotation and the magnitude of the response in reserpinized rats with ESN lesions.

324.5

EFFECTS OF MUSCIMOL (MUSC) ON D1 & D2 DOPAMINE (DA) RECEPTOR MEDIATED BEHAVIORS. L.M. Bednarz* and K.E. Asin, (SPON.: W. Montana), Neurosci.Res.Div., Pharmaceutical Discovery, Dept 47U, AP10, Abbott Labs, Abbott Pk, IL 60064.

Studies have demonstrated that the GABA agonist MUSC enhances amphetamine (AMP) and apomorphine (APO) stereotypy (STEREO), and that MUSC also enhances neuroleptic-induced catalepsy (CAT). To investigate possible MUSC interactions with the D1 and D2 DA receptor subtypes, we examined MUSC's effects on the STEREO and CAT produced by injections of D1 or D2 selective drugs.

Adult male rats were injected with MUSC (0-1.5mg/kg,sc) + either vehicle (VEH), the D1 agonist SKF38393 (5 or 20mg/kg,sc) or the D2 agonist LY171555 (.15mg/kg,sc). Beginning 15min later, each rat's behavior was rated on a 1-6 scale for 20sec every 15min for 90min. Our results indicate that MUSC enhanced the low level sniffing STEREO produced by D2 but not D1 receptor stimulation, suggesting that MUSC's effects on AMP and APO-induced STEREO rely on D2 DA receptor stimulation. We also examined the effects of MUSC (1mg/kg,ip) on the CAT produced by the D1 antagonist SCH23390 (.06 or .25mg/kg, sc), or the D2 antagonists haloperidol (.2mg/kg,sc) or metoclopramide (25mg/kg,sc). Beginning 15min later, catalepsy (bar test) was measured periodically for 90-120min. Our results suggest that muscimol potentiates both D1 and D2 DA receptor mediated catalepsy and that the mild stereotypy produced by injections of the selective DA agonists probably involves different neuronal substrates.

324.6

D2 AGONIST QUINPIROLE INDUCES PERSEVERATION OF MOTION BUT NO PERSEVERATION OF MOVEMENTS. H. Szechtman, D. Eilam and T. Golani* Dept. Biomedical Sciences, McMaster Univ., Hamilton, Ontario, CANADA L8N 3Z5.

The behaviour of rats injected with D2 agonist, quinpirole (2 mg/kg; n=10) and saline (n=10) was analyzed in terms of variables that measure motion and movements. Results indicate that quinpirole induces perseverative motion without, at the same time, inducing a perseveration of movement. Perseverative motion was characterized by repeated travel along specific trajectories in a limited portion of the environment. Lack of perseveration of movement was evidenced by the same distribution of movements as in saline-treated animals. In addition to these effects, quinpirole increased the total amount of motion and the total number of movements performed by each body segment along all dimensions. Thus, animals under quinpirole were hyperactive but free in their movements and yet, at the same time, stereotyped in motion through the environment. In light of our previous descriptions of the behavioral profiles under apomorphine and amphetamine, the present findings suggest that quinpirole induces perseveration of motion by affecting presynaptic release of dopamine. (Supported by MRC. HS is a Research Associate of the Ontario Mental Health Foundation.)

324.7

EFFECTS OF D_1 AND D_2 RECEPTOR ANTAGONISTS AND 5HT RECEPTOR AGONISTS AND ANTAGONISTS ON NEUROENDOCRINE FUNCTION IN RHESUS MONKEYS. G.R. Heninger, J.H. Krystal and A. Smith*, Dept. of Psychiatry, Yale Univ. Sch. of Medicine, New Haven, Ct. 06508

The dopamine (DA) and serotonin (5HT) systems play important roles in modulating prolactin (PR) release. The contributions of DA and 5HT receptor subtypes to PR regulation were studied in monkeys as a model for human neuroendocrine function.

METHODS: Eight male Rhesus monkeys were studied. D_1 and D_2 antagonists included: haloperidol (H) and SCH23390 (SC), 5HT agonists included: buspirone (B), 8-OHDPAT (8OH), ipsapirone (I), gepirone (G), MCPP (MC), mescaline (MS), and tryptophan (T) and antagonists: ritanserin (R) and metergoline (MI).

RESULTS: The relative potency (PO) (in uM/kg) for a 20 ng/ml prolactin increase and the percent antagonism is:

	H	B	SC	8OH	I	G	MC	ME	T
PO	.02	.2	.5	1	2	1	4	13	1000
R	0	0	-	0	-	0	50	90	18
MI	72	95	-	96	-	90	100	100	97

H plus G as well as SC plus G did not synergize, thus the D_2 and 5HT systems appear to function independently on PR release. D_2 antagonism is 50 times more potent than 5HT stimulation in increasing PR. Behavioral effects including sedation (and catatonia with H, SC and B) were marked and clear differences were observed between the drugs studied. (See J.H. Krystal, et.al. this volume).

324.8

THE ROLE OF SPECIFIC DOPAMINE RECEPTOR SUBTYPES IN d-AMPHETAMINE (AMPH) DISCRIMINATION F.L. Smith* and W.H. Lyness. Dept. of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

In male Sprague-Dawley rats trained to discriminate 1.0 mg/kg of AMPH from saline, substitution with the D-2 agonist quinpirole (QUIN) (0.1-2.0 mg/kg) produces drug-lever responding, whereas the D-1 agonist SKF 38393 (0.3-10.0 mg/kg) elicits only saline-lever responding. Combining either QUIN (0.05 - 0.5 mg/kg) or SKF 38393 (0.5-10.0 mg/kg) with 0.3 mg/kg AMPH results in dose-related increases in drug-lever responding. Conversely, the D-1 antagonist SCH 23390 (0.02-0.1 mg/kg) antagonizes the discrimination produced by 0.7 mg/kg AMPH. Additional studies examined the effect of DA drugs on the discrimination produced by substituting QUIN for AMPH. SKF 38393 (0.5 mg/kg) fails to increase the discriminative cues produced by either 0.05 or 0.2 mg/kg QUIN. Similarly, SCH 23390 (0.01-0.1 mg/kg) fails to antagonize the drug-lever responding produced by either 0.2 or 0.5 mg/kg QUIN. Haloperidol (0.02-0.2 mg/kg) antagonizes responding produced by QUIN substitution. The AMPH experiments indicate that stimulating D-2 receptors yields drug-lever responding, and suggests that D-1 and D-2 receptors may functionally interact to alter discrimination behavior. QUIN substitution shows insensitivity to D-1 agonism/antagonism. Partially supported by NIDA grant DA-02997.

324.9

D_2, BUT NOT D_1, RECEPTORS IN THE MPOA DECREASE EJACULATORY THRESHOLD IN MALE RATS. E. Hull, R. Warner*, T. Bazzett*, R. Eaton*, E. Pehek, J. Thompson* and M. Swain*. Dept. of Psychology, SUNY at Buffalo, Amherst, NY 14260.

We have previously shown that the classic dopamine (DA) agonist apomorphine (APO), injected into the medial preoptic area (MPOA) of male rats, facilitated several copulatory measures, including increased ejaculations per test. The DA antagonist cis-flupenthixol (FLU) blocked APO's facilitation and, in higher doses, impaired copulation. To determine whether DA facilitation of copulation is mediated by D_1 or D_2 receptors, we injected either the D_2 agonist LY163502, the D_1 agonist SKF82526, or a combination of the two into the MPOA immediately before sexual behavior tests.

In Exp. 1, 1 ug LY reduced the number of intromissions preceding ejaculation. In Exp. 2, .2 or 2 ug SKF did not affect copulation; 20 ug SKF reduced the number of ejaculations. In Exp. 3, co-administration of 1 ug LY + 2 ug SKF did not summate to facilitate behavior; both LY alone and LY+SKF decreased intromissions preceding ejaculation. In Exp. 4, 10 ug FLU failed to block the reduction in intromissions preceding ejaculation caused by 1 or 10 ug LY.

In summary, the D_2 agonist LY163502 decreased the number of intromissions preceding ejaculation (ejaculatory threshold). This effect was not enhanced by co-administration of the D_1 agonist SKF82526, nor blocked by co-administration of the DA antagonist cis-flupenthixol.

324.10

INTERACTION OF D1 AND D2 RECEPTORS IN THE EXPRESSION OF SENSORIMOTOR DEFICITS IN MPTP-TREATED MICE.

F.B. Weihmuller*, J.P. Bruno, and M. Hadjiconstantinou, (SPON: S. Tjioe), Depts. of Psychology and Pharmacology, The Ohio State University, Columbus, Ohio 43210.

MPTP is a useful tool for understanding the etiology and pharmacology of parkinsonism. We recently reported that MPTP-treated mice exhibit motor impairments and sensory neglect after small doses (0.2 mg/kg) of haloperidol that have no effect in control animals. To determine the relative contribution of dopamine (DA) D1 and D2 receptors to this effect we examined the ability of selective DA antagonists, SCH 23390 (D1) and 1-sulpiride (D2), to induce sensorimotor deficits. Small to moderate doses of SCH 23390 (0.2-0.5 mg/kg) induced only motor deficits and only in MPTP-treated mice; whereas a higher dose (1.5 mg/kg) caused motor and sensory impairments in both groups of mice. MPTP-treated mice were supersensitive to the motor, but not to the sensory, effects of high doses of 1-sulpiride (100-150 mg/kg). Moreover, combined subthreshold doses of SCH 23390 (0.2 mg/kg) and 1-sulpiride (50 mg/kg) induced pronounced sensorimotor deficits in MPTP, but not saline-treated mice. These findings suggest that D1 and D2 receptors contribute to different aspects of sensorimotor behavior and their interaction is necessary for the expression of deficits in DA-depleted mice.

324.11

FACILITATION OF SEXUAL AROUSAL IN MALE AND FEMALE RHESUS MONKEYS (*Macaca mulatta*) BY THE DOPAMINE (D2) AGONIST, LY163502. G.A. Davis, R.W. Goy*, S. Baum* and J. Johnson* Wisc. Reg. Primate Res. Ctr., University of Wisconsin-Madison, WI 53715.

Dopamine agonists have long been reported to facilitate male sexual activity in humans (H.P. Vogel & R. Schiffter, *Pharmacopsychiatria* 16:107, 1983) as well as in rats. The clearest demonstration of this action has been made recently with a new compound, LY163502 (Eli Lilly & Co.), which is a highly potent and selective agonist at D2 receptors. This drug facilitates gender specific sexual behavior in both male and female rats at very low doses (Foreman & Hall, *Psychopharmacol.*, 91:96 1987; *J. Neural Transm.* 68:153, 1987). Male and female rhesus were tested with this drug in a confined partner paradigm, in which the test animal was allowed to view a standardized stimulus animal of the opposite sex but not to contact it physically. The test was carried out for a 30 min period, and indicators of sexual arousal, such as erection or presentation, as well as other behaviors, were scored in 10 sec blocks.

At doses of 10 or 25 μg/kg, LY163502 caused a 5-fold increase over saline controls in the erection rate of male animals ($p<0.001$). A dose of 1 μg/kg was ineffective. Purselip (a putative courtship signal), groom solicit and feeding were also elevated in rate, but only at 25 μg/kg. The increases in erection and purselip, in contrast to those in feeding and groom solicit, were completely dependent on the presence of a female stimulus animal. Males that had been castrated at birth were also tested in this procedure, at a dose of 25 μg/kg. These animals had received repeated testosterone injections at various times, but all treatments ceased at least one year before the present experiment. The castrates responded behaviorally much as the intact males had, but with substantially lower erection rate and quality (on a 1 to 3 scale).

Female subjects, tested with a male stimulus animal, also displayed increased sexual behaviors when treated with LY163502 at 10 or 25 μg/kg. Solicitation signals such as hand slap, along with presentation, were significantly elevated in rate. The increases did not occur in the absence of the male stimulus animal. These results are consistent with the hypothesis that dopamine plays an important role in regulating sexual arousal in both male and female primates.

Supported by grants from Eli Lilly and Co. and NIH RR00167.

324.12

EFFECTS OF D1 AND D2 AGONISTS ON THE ACQUISITION OF RESPONDING FOR CONDITIONED REWARD (CR). R.J. Beninger. Dept. of Psychol., Queen's University, Kingston, K7L 3N6, Canada.

A stimulus that is repeatedly paired with reward can acquire the ability to act as a reward in its own right, becoming a CR. In a Skinner box with two levers, the acquisition of responding on one lever for CR is differentially affected by direct and indirect-acting DA agonists, amphetamine enhancing responding on the CR level in a dose-dependent manner and apomorphine enhancing responding on both. To determine the contribution of D1 and D2 receptors to this phenomenon, the effects of quinpirole (0.01-5.0 mg/kg), bromocriptine (0.05-10 mg/kg) and SKF 38393 (0.1-10 mg/kg) were assessed with an independent group receiving each dose. Whereas SKF 38393 was without significant effect, the two D2 agonists in a dose-dependent manner produced an amphetamine-like effect, enhancing responding on the CR lever but not the other one. The dopaminergic nature of the bromocriptine effect was confirmed by the observation that pimozide (0.4 mg/kg) shifted the dose-response function to the right. The differential effects of apomorphine and D2 agonists suggests that simultaneous direct stimulation of D1 and D2 receptors by the nonspecific agonist led to the loss of stimulus control by the CR in a manner that did not occur with direct stimulation of only D2 receptors. (Funded by the Natural Sciences and Engineering Research Council of Canada.)

324.13

THE EFFECTS OF SELECTIVE DOPAMINE D1 OR D2 RECEPTOR ANTAGONISTS ON THE ESTABLISHMENT OF AGONIST-INDUCED PLACE CONDITIONING. D.C. Hoffman and R.J. Beninger. Dept. Psychol., Queen's University, Kingston, Canada.

The reinforcing effects of dopaminergic agonists have been demonstrated using the place conditioning procedure. After receiving several pairings of a drug injection in one side of a box and not the other, the undrugged animal subsequently demonstrates a preference for the drug-paired side. At least two different dopamine receptor subtypes have been identified and recent studies have investigated the ability of selective D1 or D2 receptor agonists to produce place conditioning. The D2 agonist, quinpirole produced a place preference whereas the D1 agonist, SKF 38393 produced a place aversion. To further assess the contribution of D1 and D2 receptors to these place conditioning effects, the D1 antagonist, SCH 23390 or the D2 antagonist, metoclopramide was tested with an effective dose of either the nonselective agonist, amphetamine or the subtype-specific agonists, quinpirole or SKF 38393 in male Wistar rats. SCH 23390 and metoclopramide were effective in blocking amphetamine-induced place preference and SKF 38393-induced place aversion. At lower doses, the D1 or D2 antagonist blocked the place preference induced by quinpirole, however, higher doses were not effective. Interestingly, the high dose of the D2 antagonist, but not the D1 antagonist, produced a place preference on its own that approached significance. In general, these data suggest that stimulation of both receptor subtypes is necessary for the establishment of place conditioning with amphetamine, SKF 38393 or quinpirole. (Funded by Natural Sciences and Engineering Research Council).

324.14

DOPAMINE D1 AND D2 ANTAGONISTS DIFFERENTIALLY BLOCK CONDITIONED LOCOMOTION BASED ON NONSELECTIVE, D1 OR D2 AGONISTS. E.J. Mazurski and R.J. Beninger. Dept. Psychol., Queen's University, Kingston, Ontario, K7L 3N6 Canada.

Recent evidence suggests that two receptor subtypes exist for dopamine (DA), termed D1 and D2, and drugs are now available that preferentially stimulate or block each type. Classical conditioning of locomotor activity using nonselective DA agonists as the unconditioned stimuli has frequently been demonstrated. Thus, after pairings of (+)-amphetamine (AMPH) with a specific environment rats showed enhanced activity when later given saline and placed there in comparison to a group with the same drug history, but noncontingent drug-environment pairings. Experiment 1 examined conditioned activity with AMPH (2.0 mg/kg), the D1 agonist SKF 38393 (SKF, 10.0 mg/kg), and the D2 agonist quinpirole (QUIN, 2.5 mg/kg). Two groups of 12 rats for each drug received either the drug or saline paired with automated chambers that assessed horizontal and vertical activity for nine 2-h sessions. After every third conditioning session tests were given where both groups received saline. During pairings all drugs produced hyperactivity. On saline tests the paired AMPH group showed more horizontal and vertical activity than its control. The SKF group showed only conditioned horizontal activity and the QUIN group showed only conditioned vertical activity. Experiment 2 examined the effects of co-administration of the D1 antagonist SCH 23390 (0.05 mg/kg) or the D2 antagonist metoclopramide (10.0 mg/kg) with AMPH or QUIN, and METO with SKF during conditioning. The D1 antagonist blocked conditioning only with AMPH. The D2 antagonist blocked conditioning only with QUIN. Thus, selective agonists produce conditioned activity that is blocked by selective antagonists. (Funded by NSERC.)

PHARMACOLOGY OF SYNAPTIC TRANSMISSION II

325.1

ELECTPOPHYSIOLOGICAL EVIDENCE THAT ADENOSINE REQUIRES MAGNESIUM FOR ITS INTERACTION AT THE A1 ADENOSINE RECEPTOR J.T. Bartrup* and T.W. Stone. Department of Physiology, St George's Hospital Medical School, London SW17 0RE, UK.

Binding to the A1 adenosine receptor has been shown to be influenced by magnesium ions (Mg). Removal of Mg also induces epileptiform activity in brain slices. By studying CA1 orthodromically evoked population potentials in the rat hippocampal slice, we have looked at the effect of varying Mg concentrations on synaptic inhibition by adenosine.

In standard 1.2mM Mg, adenosine depressed the population potential with an IC50 of 37±3.4μM (n=30). When Mg was omitted from the ACSF, the depression by adenosine was significantly reduced (IC50 124.6±15μM, n=5). A similar loss of potency was observed with 2-chloroadenosine.

Removal of Mg increased the potential size (12±1.6%, n=13) but this did not account for the loss of adenosine potency. Reducing the calcium (Ca) from 2.5mM to 1.2mM reduced the potential size, but failed to restore adenosine potency. Addition of cobalt (500μM), however, did restore the adenosine inhibition.

In 0Mg/ low Ca, adenosine (50μM) occasionally gave a large increase in potential size that could be rapidly reversed by theophylline (50μM). This response could also be elicited by N-ethylcarboxamidoadenosine, 2-phenylamino-adenosine and N-(9H-fluoren-9ylmethyl)adenosine, selective A2 receptor agonists.

Supported by the Science & Engineering Research Council.

325.2

BACLOFEN INHIBITS GLUTAMIC ACID RELEASE FROM PRIMARY CEREBELLAR GRANULE CELLS THROUGH A PERTUSSIS TOXIN SENSITIVE GUANINE NUCLEOTIDE COUPLING PROTEIN. M. Ulivi*, W. Wojcik and E. Costa, (SPON: J. Cohen). FGIN, Georgetown Univ., Washington, D.C. 20007.

Primary cerebellar granule cell cultures were used to study the effects of the $GABA_B$ receptor agonist, baclofen, on the release of glutamic acid used by these cells in neuron to neuron signaling. Glutamate was separated from other substances by strong cation exchange HPLC and detected by post-column derivatization with OPA before fluorescence detection. During a 1 min. exposure to 60 mM K^+, the glutamate detected in the incubation buffer increased by 6 to 8 fold. This release of glutamate was calcium dependent. Baclofen attenuated the 60 mM K^+ evoked release in a concentration dependent manner (EC_{50} 20 uM) and did not affect the basal release of glutamate. Maximal concentrations of baclofen inhibited only 50% of the K^+ evoked release. In cultures that were treated with pertussis toxin (1 ug/ml, 15 hrs), the concentration dependent inhibition of glutamate release by baclofen was attenuated by 69%.

325.3

EVIDENCE THAT THE DEPRESSION OF RECURRENT INHIBITION FOLLOWING TETANIC STIMULATION IN THE RAT DENTATE GYRUS IS MEDIATED BY $GABA_B$ RECEPTORS. D.D. Mott*, D.V. Lewis, A.C. Bragdon and W.A. Wilson. Depts. of Pharmacology, Physiology, Pediatrics (Neurology) and Medicine (Neurology), Duke University and Veterans Administration Medical Centers, Durham, NC 27710.

A variety of mechanisms have been proposed to explain the depression of synaptic inhibition which follows repetitive firing. We have shown that $GABA_B$ receptor activation by baclofen reduces recurrent inhibition (RI) in the rat dentate gyrus by an action on the inhibitory interneuron (Mott and Bragdon, 1987). A similar effect has been demonstrated in cultured hippocampal neurons (Harrison et al., 1988). Here we report that tetanic mossy fiber stimulation induces transient depression of RI in the rat dentate gyrus, and that the $GABA_B$ receptor antagonist phaclofen can prevent this depression.

RI was studied in hippocampal slices from 80-200 g male Sprague-Dawley rats. Perforant path (PP) stimulation evoked an EPSP and a population spike (PS) in the dentate gyrus granule cell layer. RI was induced by a single antidromic stimulus to the mossy fibers (MF) 5 msec before PP stimulation. RI was quantified as the % reduction in the PP-evoked PS. Phaclofen (24 mM) was pressure-ejected (10 msec, 30 psi) from a pipette placed near the recording electrode in the granule cell layer. Phaclofen reduced the PP-evoked PS but had no consistent effect on RI.

Tetanic MF stimulation (50 Hz, 1-2 sec) reduced RI by about 50% (N=3) for up to 1000 msec after the stimulus train and converted the PP-evoked response to one containing multiple PS's during this period. Phaclofen reversed this stimulus train-induced loss of RI. After a stimulus train, in the presence of phaclofen, RI was increased by up to 60%. In addition, the PP-evoked PS was reduced, and no secondary PS's developed. All effects of phaclofen were reversible.

These results suggest that GABA released during repetitive firing can suppress $GABA_A$-mediated RI by acting at $GABA_B$ receptors on inhibitory interneurons.

Supported by NIH grants GM07184, NS 22170, NS 17771, and the VA.

325.4

PHARMACOLOGICAL CHARACTERIZATION OF ELECTROPHYSIOLOGICAL EFFECTS OF NICOTINE IN MOUSE HIPPOCAMPUS. R. K. Freund, D. A. Jungschaffer*, and A. C. Collins. Instit. for Behav. Genetics, Univ. of Colorado, Boulder, CO 80309

Previous studies have indicated that nicotine (Nic) induces a concentration-dependent increase in the hippocampal CA1 population spike (PS) and the induction of secondary spiking. In an attempt to characterize the receptor(s) responsible for these effects, a series of nicotinic and muscarinic antagonists were tested for their effects on CA1 PS's from DBA mice. D-tubocurarine (D-TC; 10-100 uM) and atropine (100-400 uM) increased the PS and induced secondary PS's. -Bungarotoxin (10-160 uM) had similar effects at early exposure times (< 10 min.), but then responses decreased steadily and PS's could not be recovered upon washing. Mecamylamine (Mec; 0.8-3.2 mM) gave inhibitory effects during exposure and excitatory effects after washing, but no secondary PS's were observed. Hexamethonium (Hex; 3.2 mM) had little or no effect on CA1 PS's.

Several of these antagonists have been tested for the ability to inhibit effects of Nic (800 uM), at antagonist concentrations below those which elicit effects of their own. Mec was effective for inhibiting effects of Nic, whereas Hex was not. Preliminary data indicate that D-TC is also ineffective for blocking responses to Nic. These results suggest that the pharmacology of nicotinic receptors in brain is different from that found in the periphery.

(Supported by R. J. Reynolds Tobacco Co.)

325.5

ARGIOTOXIN-636 BLOCKS GLUTAMATE-MEDIATED SYNAPTIC TRANSMISSION AND RESPONSES TO EXOGENOUS GLUTAMATE IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. C.L.Cox, J.H.Ashe, and M.E.Adams. University of California, Riverside, CA 92521.

Synthetic argiotoxin-636 (AR-636), a toxin identified from orb weaver spider venom (Adams et.al. Biochem. Biophys. Res. Comm., 148: 678-683, 1987), was tested for effects on excitatory synaptic transmission in CA1 pyramidal neurons of the in vitro rat hippocampus. Microtopical application of AR-636 (5nM-50nM) resulted in a dose dependent suppression of the amplitude of the dendritic field EPSP recorded from the stratum radiatum and the population spike amplitude recorded from the stratum pyramidale of CA1. In contrast, the amplitude of the antidromic spike was not affected by exposure to AR-636. The maximum effect of AR-636 on the amplitude of the EPSP occurred at 15-25 minutes following application. Reversal of AR-636 induced suppression of the field EPSP was inversely related to concentration. Cellular discharge elicited by pressure ejection of l-glutamate (0.2M) was abolished by AR-636 (15-25nM), whereas responses to pressure ejection of l-aspartate (0.2M) were not significantly affected by AR-636. These data suggest that AR-636 acts as a selective antagonist of glutamate-mediated synaptic transmission in rat hippocampus.

325.6

EFFECT OF DESIPRAMINE AND AMPHETAMINE ON NORADRENERGIC SYNAPTIC TRANSMISSION: IN VIVO STUDIES IN THE RAT DORSAL HIPPOCAMPUS. O. Curet and C. de Montigny. Dept. of Psychiatry, McGill University, Montreal, Canada H3A 1A1.

The present study was undertaken to determine the effects of desipramine (DMI) and amphetamine (AMPH) on noradrenergic (NE) synaptic transmission. CA_3 hippocampus pyramidal neurons were recorded with five-barreled micropipettes. The central barrel was filled with 2 M NaCl and side barrels with NE (0.05M in 0.2M NaCl; pH 4), acetylcholine (0.02M in 0.2M NaCl; pH 4) and 2M NaCl. A bipolar stimulating electrode was positioned in the locus coeruleus (LC). 150 square pulses were delivered at 1 Hz with an intensity of 800 uA. The degree of suppression of pyramidal neurons firing activity was quantified from peristimulus time histograms.

DMI (0.5-5 mg/kg, i.v.) and AMPH (0.25-5 mg/kg, i.v.) both decreased the effect of the LC stimulation and increase the duration of the response of the same neurons to the microiontophoretic application of NE. The subsequent intravenous injection of idazoxan, an α_2-adrenergic antagonist, restored the effectiveness of the LC stimulation.

It is concluded that the acute administration of either DMI or AMPH, by increasing the concentration of NE in the synaptic cleft, results in a decreased effectiveness of the LC stimulation due to an increased activation of terminal α_2-adrenergic autoreceptors.

325.7

DIFFERENTIAL EFFECTS OF D-AMPHETAMINE MEASURED INTRACELLULARLY IN HIPPOCAMPAL DENTATE GRANULE NEURONS IN VITRO FROM PREPUBERTAL AND POSTPUBERTAL RATS. S.S. Jahromi*, P.L. Carlen and C.E. Niesen. Playfair Neuroscience Unit, Addiction Research Foundation, Departments of Physiology and Medicine (Neurology), The Toronto Western Hospital, University of Toronto, Toronto, Ontario, M5T 2S8.

The effects of d-amphetamine (10-100 uM) on hippocampal dentate granule neurons in vitro were compared in pre- and postpubertal male Wistar rats using intracellular electrophysiological recording techniques.

Amphetamine, either drop-applied or perfused, caused the following:
1) A prolonged depolarization (5 to 25 mV) with increased spontaneous activity 10/13 on neurons of postpubertal rats; 2) A prolonged hyperpolarization (-3 to -8 mV) in 9/10 of prepubertal rats. All other results are similar for neurons of either pre- or postpubertal rats; 3) Marked attenuation of the post-spike train long lasting afterhyperpolarization; 4) Decreased spike frequency adaptation during a 600 msec depolarizing current pulse; 5) Decreased spike threshold to injected current even when the membrane potential was returned to predrug control levels; 6) Increased EPSP amplitude, sometimes giving rise to multiple spikes.

These drug effects were only slightly reversed with over one hour of washout.

In prepubertal neurons from rats, the actions of hyperpolarization along with decreased spike frequency adaptation and increased EPSPs may be relevant to the use of amphetamine in the attention deficit syndrome.

Supported by the Hospital for Sick Children Foundation and MRC.

325.8

ANTIEPILEPTIFORM EFFECTS OF CYCLIC AMP IN THE CA3 REGION OF RAT HIPPOCAMPAL SLICES. *S. A. Helekar* and F. J. Lebeda* (SPON: F. Pirozzolo), Program in Neuroscience and Section of Neurophysiology, Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.

We have observed that cyclic AMP (cAMP 5-80μM) produces a reversible decrease in frequency of extracellularly recorded interictal discharges induced by bethanechol (40μM) and theophylline (10-20μM) in the CA3 region of rat hippocampal slices. Since it has also been observed that 5'-AMP, adenosine and its analogs produce a similar effect, we have tested the hypothesis that cAMP acts indirectly by being converted extracellularly to adenosine by two enzymatic steps catalysed by a phosphodiesterase (PDE) and a 5'-nucleotidase (5NT). This hypothesis predicts the following: 1) blocking the breakdown of cAMP would attenuate its effects and 2) nonhydrolysable analogs of cAMP would have minimal or no reversible effects.

In contrast to the first prediction, the effect of cAMP is potentiated by coapplication of α,β-methyleneadenosine 5'-diphosphate (AMP-CP 8-16μM) or 3-isobutyl-1-methylxanthine (IBMX 10-20μM), a 5NT and a PDE inhibitor (with an additional adenosine receptor blocking action) respectively. Control experiments with these compounds suggest that they do not, by themselves, have any antiepileptiform action. Furthermore, nonhydrolysable derivatives of cAMP, dibutyryl- (10-25μM) and 8-bromo-cAMP (5-10μM) are as potent as cAMP in their discharge suppressant action. These data are not consistent with the idea that the antiepileptiform action of exogenously applied cAMP is mediated solely by metabolic conversion to adenosine and suggest an additional, possibly a direct mode of action involving extracellular sites for cAMP and its first metabolic product, 5'-AMP.

(Supported by USAMRDC contract DAMD17-86-C-6029, AFOSR85-0178 and NIH grant NS11535)

325.9

EFFECTS OF AN ANESTHETIC ALPHA-2 ADRENERGIC AGONIST, DEXMEDETOMIDINE, ON RAT HIPPOCAMPAL CA 1 NEURONS. V.A.Doze, M.B.MacIver, M.Maze, J.J.Kendig (SPON: H.Schulman). Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5117.

Dexmedetomidine, a highly specific alpha-2 adrenergic receptor agonist, produces a behavioral state analogous to general anesthesia in the rat. The anesthetic effect is stereospecific and blocked by alpha-2 blocking agents. The present study examined electrophysiological responses to dexmedetomidine in a rat hippocampal slice preparation.* Synaptically evoked field potentials were recorded from CA 1 neurons by standard extracellular techniques. Perfusion of dexmedetomidine (10-100 nM) produced reversible concentration-dependent increases in both population spike (PS) and field EPSP amplitudes (110-200% of controls). Higher concentrations of dexmedetomidine (>1 uM) depressed both PS and EPSP. This apparent biphasic response differs from the responses (depression of PS and EPSP) observed with volatile general anesthetics in this preparation, but resembles to some extent the effects produced by pentobarbital. *Approved by Stanford Panel on Laboratory Animal Care. Supported by NIH Grant NS13108 to JJK.

325.10

EXTRACELLULAR CESIUM INCREASES THE AMPLITUDE OF CA1 DENDRITIC RESPONSES EVOKED BY REPETITIVE STIMULATION IN HIPPOCAMPAL SLICES. D.B.Macek*, J.P.Harris*, and A.L.Padjen, Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1Y6.

We have compared the effect of extracellular cesium ($[Cs]_o$, 3 mM) on the responses of rat hippocampal neurons to different patterns of repetitive stimulation. D(-)-2-amino-5-phosphonovalerate (APV, 40 μM) was present in ACSF to prevent potentiation. Dendritic field potentials of the CA1 region were evoked by unpatterned stimuli to the stratum radiatum (100 pulses at 50 Hz for 2 sec), resulting in a prolonged slow depolarization that was double the amplitude of the short depolarizing responses to patterned stimuli (100 pulses grouped in 10 bursts of 10 pulses @ 100 Hz for 2 sec). In the presence of extracellular cesium (3 mM) both responses increased in amplitude, with unpatterned >> patterned.

These results are consistent with the hypothesis that $[Cs]_o$ facilitates transmitter release, possibly by blocking anomalous rectifier in terminals. /Supported by MRC/

325.11

CNQX BIOCKS EXCITATORY SYNAPTIC TRANSMISSION, QUISQUALATE INDUCED CURRENTS AND BURSTS IN HIPPOCAMPAL SLICES. R.S. Neuman, Y. Ben-Ari, M. Gho and E.Cherubini. INSERM U29, 123 Bv. De Port-Royal, Paris, France.

The action of CNQX, a purported quisqualate antagonist (Honore et al. 1987, Soc. Neurosci. Abstr.), was studied on hippocampal slices in vitro. Bath application of CNQX (2-4uM) rapidly and reversibly blocked the Schaffer collateral and mossy fibre evoked e.p.s.p. The blockade was not associated with changes in membrane potential, membrane resistance or spike accommodation. In addition, the fast and slow GABA mediated inhibition evoked by mossy fibre stimulation was not altered by CNQX. CNQX (2-3uM) also blocked spontaneous and evoked bursts in the CA3 region induced by NMDA and kainate, as well as the persistent bursts following kainate washout, the latter with 0.5uM CNQX. The effects of CNQX were also tested on the currents induced by excitatory amino acids in the presence of TTX using the single electrode voltage clamp technique. Inward currents induced by quisqualate (10uM), kainate (200nM) and NMDLA (20uM) were reduced to 32±4%, 78±17 and 66±11 of control respectively by 10uM CNQX.

From our observations we suggest that the transmitter for the e.p.s.p. evoked by Schaffer collateral and mossy fibre stimulation acts on the quisqualate type receptor and furthermore that this same receptor plays a substantial role in burst generation.

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325.12

DEPRESSION OF SYNAPTIC TRANSMISSION BY ω-CONOTOXIN IN THE RAT HIPPOCAMPAL SLICE. *D.B. Jaffe* and D. Johnston*, Program in Neuroscience, Baylor College of Medicine, Houston, Texas 77030.

The release of neurotransmitters from the presynaptic terminal of a neuron is dependent on calcium ions that enter through voltage-sensitive calcium channels in the plasma membrane. ω-conotoxin GVIA (ω-CTX) is an irreversible inhibitor of synaptic transmission in a number of peripheral synapses, and it binds to synaptic plasma membranes from rat brain. ω-CTX has been shown to block both N and L-type channels while having no long-lasting effect on the T-type channel, suggesting that the N and/or L channels are responsible for regulation of synaptic transmission. We studied the effects of ω-CTX on synaptic transmission in the *in vitro* hippocampal slice preparation at both the Schaffer collateral (SC)-CA1 and mossy fiber (MF)-CA3 synapses. Transverse rat hippocampal slices, 400 μm thick, were maintained in artificial CSF (aCSF) at 34-35°C in an interface chamber. Population excitatory postsynaptic potentials (pEPSPs) were recorded either in stratum pyramidale of CA1 or in stratum lucidum of CA3b. ω-CTX (5 μM) was applied as a microdrop to the surface of either stratum radiatum of CA1 or stratum lucidum of CA3. Application of 5 μM ω-CTX to the dendritic field of CA1 or CA3 irreversibly decreased SC evoked pEPSPs 39 ± 6% (n=3) and MF evoked pEPSPs 34 ± 5% (n=6), respectively, indicating a net decrease in synaptic transmission. Microdrop application of control vehicle had no effect on synaptic transmission at either the SC-CA1 (n=3) or MF-CA3 (n=4) synapses. The decrease in synaptic transmission in the hippocampus upon administration of ω-CTX to the major excitatory inputs of both the CA1 and CA3 regions suggests involvement of the N- and/or L-type calcium channel currents in the regulation of synaptic transmission at these sites. Depression of synaptic transmission by ω-CTX was less than 50%, however, which may indicate a role for the T-type or a non ω-CTX-sensitive calcium channel subtype in synaptic transmission. Nonuniform exposure of synaptic terminals to ω-CTX could also be a factor in the magnitude of the changes observed. Modulation of the properties of these channels may alter the efficacy of synaptic transmission and may be involved in the mechanism of long-term potentiation. (NIH grants NS11535 & HL31164 and AFOSR 85-0178)

325.13

NMDA CHANNEL ACTIVATION MEDIATES PROLONGED PAIRED PULSE DEPRESSION OF POPULATION SPIKE AMPLITUDE IN RAT HIPPOCAMPUS. R.S. Goldman and C.F. Stevens. Section of Molecular Neurobiology and Department of Neurology, Yale University School of Medicine, New Haven, CT. 06510.

The effect of varying magnesium (Mg) concentrations on the population spikes of paired pulses was studied in area CA1 of rat hippocampal slices. Reduction in Mg concentration resulted in a dose dependent depression of the ratio of the spike amplitude of the second pulse to that of the first pulse. This reduction lasted for hundreds of milliseconds, and was not influenced by picrotoxin, implying that this effect was not related to an effect of Mg on inhibition. Across different Mg concentrations there was a correlation between the amount of inhibition of the second population spike and the value of the field excitatory postsynaptic potential (EPSP) at the corresponding time interval. D-amino-phosphonovaleric acid, a specific blocker of the N-methyl-D-aspartate (NMDA) channel blocked the inhibition of the second pulse. Together with the dependence upon the Mg concentration, this implies involvement of the NMDA channel in mediating the depression. The field EPSP was not depressed to a comparable extent as the population spike, implying that the paired pulse depression was due primarily to a change in the excitability of the postsynaptic cell. Evoked responses following a spontaneous firing showed a similar inhibition.

In terms of possible mechanisms, we favor the view that prolonged depolarization due to NMDA channel activation (unmasked by low Mg) results in prolonged sodium channel inactivation. However, we cannot exclude the possibility that transmission of the synaptic current to the soma is impaired due to shunting of current by activated channels in the dendrites.

325.14

DO GENERAL ANESTHETICS HYPERPOLARIZE MAMMALIAN CNS NEURONS? J.J.Kendig and M.B.MacIver. Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5117.

Unitary theories of anesthesia propose that all general anesthetics share a common mechanism. One suggested common action is hyperpolarization of CNS neurons through increase in potassium conductances. The present study examined whether inhalation anesthetics (halothane, enflurane, isoflurane, methoxyflurane) share this action. Synaptically evoked responses, resting potential and conductance were measured intracellularly in CA 1 neurons of rat hippocampal slices.* Agents were administered via the surface gas stream at equivalent anesthetic concentrations (1 MAC). Only halothane produced hyperpolarization (3-5 mV) accompanied by a conductance increase (10-15%). Isoflurane depolarized CA 1 neurons and decreased conductance. Enflurane also decreased conductance, but produced biphasic (hyperpolarizing/depolarizing) actions. All agents blocked the synaptically evoked population spike but were variably effective in depressing EPSP amplitude, in the order methoxyflurane > isoflurane ≥ enflurane >> halothane. These results do not support a unitary theory of anesthesia; instead, there appear to be agent-specific actions at multiple membrane sites. *Protocol approved by Stanford Laboratory Animal Care Panel. Supported by NIH Grant NS13108 to JJK.

325.15

SYNAPTICALLY EVOKED SPIKE INITIATION IN DENDRITES OF CA 1 HIPPOCAMPAL NEURONS. M.B.MacIver and J.J.Kendig. Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5117.

Both dendritic and somatic sites have been proposed as the spike initiation zone in synaptically activated hippocampal CA 1 neurons. The present study used fixed and roving extracellular electrodes (ACSF filled, 0.5-2 M ohm) and intracellular electrodes (K acetate filled, 80-120 M ohm) in rat hippocampal slice.* Bipolar stimulating electrodes were placed in s. radiatum. Field potentials were monitored at various levels along the axis of CA 1 neuron apical dendrites. At low stimulus intensities a negativity, corresponding to the positive EPSP recorded in the somatic region, reached maximum amplitude at the level of the activated fibers in s. radiatum. At stimulus intensities which produced a half maximal population population spike, a second larger but briefer negativity was superimposed on the first, with maximum amplitude 150-200 u closer to the somatic layer, but still well within the dendritic layer in s. radiatum. This second wave occurred 0.5 to 2 ms before the population spike at the somatic level or the action potential recorded intracellularly in the soma. These results support a dendritic site for synaptically evoked spike initiation in apical dendrites of CA 1 neurons. *Protocol approved by Stanford Laboratory Animal Care Panel. Supported by NIH Grant NS13108 to JJK.

325.16

APV BLOCKS ENFLURANE-INDUCED BURST DISCHARGE OF HIPPOCAMPAL CA 1 NEURONS. D.L.Tauck, M.B.MacIver and J.J.Kendig. Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5117.

Enflurane is the only clinical general anesthetic which produces seizure activity in the hippocampal cortical EEG. The present study investigated cellular mechanisms underlying this action in the rat hippocampal slice.* Synaptically evoked responses were recorded from CA 1 neurons by standard intracellular and extracellular techniques. Enflurane was administered via the surface gas stream. An anesthetic concentration of 2.0 vol % (1 MAC) hyperpolarized the cells (~5 mV), decreased conductance, depressed both population and single cell EPSP amplitudes (30% of control) and blocked synaptically evoked discharge. Threshold to intracellular current injection was unchanged. At 3-4 vol % enflurane induced high frequency burst discharges closely resembling seizure activity *in vivo*. Depolarization and increased conductance accompanied burst discharges. The NMDA receptor antagonist APV blocked enflurane-induced burst discharge with little effect on threshold, resting potential or EPSP amplitude. An increase in NMDA-gated calcium currents accounts for enflurane-induced burst discharge, depolarization and conductance increase. *Protocol approved by Stanford Laboratory Animal Care Panel. Supported by NIH Grant NS13108 to JJK.

325.17

INFLUENCE OF ETHANOL ON MUSCARINIC RECEPTOR-G PROTEIN INTERACTIONS IN RAT BRAINSTEM. R.S. Aronstam and T.K. Narayanan (SPON: B.B. Gallagher), Dept. Pharmacol. & Toxicol., Medical College of Georgia, Augusta, GA 30912

The influences of alcohols on 1) agonist binding to muscarinic acetylcholine receptors and 2) receptor coupling to guanine nucleotide-dependent transducer proteins (G proteins) were determined in membranes prepared from rat brainstem. The effects of ethanol on carbamylcholine binding (determined in competition studies with [^{3}H]methylscopolamine) were temperature-dependent: At 4° the majority of receptors were in a high affinity conformation and ethanol increased agonist affinity; at 37° the majority of receptors were in a low afffinity conformation and ethanol further decreased binding affinity. At 25°, ethanol inhibited the binding of 2 nM [^{3}H]oxotremorine-M to high affinity receptors (IC50 = 0.5±0.1%). The guanine nucleotide sensitivity of [^{3}H]Oxo-M binding (an indication of receptor interaction with G proteins) was greatly decreased by ethanol (EC50 = 0.6±0.2%). Guanine nucleotide sensitivity was also decreased by a number of other alcohols with the following order of potency: n-octanol>n-butanol>ethanol>isopropanol=methanol. In this action, alcohols resembled anesthetics (Biochem. Pharm. 26:1201, 1987), raising the possibility that interference with receptor-G protein coupling underlies depression of synaptic transmission caused by a variety of agents.

Supported by GM-37948 and AA-07698.

325.18

HALOTHANE INHIBITION OF ACETYLCHOLINE-STIMULATED, PERTUSSIS TOXIN-SENSITIVE GTPASE ACTIVITY IN RAT BRAIN. B.L. Anthony, R.L. Dennison, T.K. Narayanan and R.S. Aronstam. Depts. of Pharmacol. & Toxicol. and Anesthesiol., Medical College of Georgia, Augusta, GA 30912.

We have demonstrated that anesthetics interfere with the interactions of muscarinic acetylcholine receptors with transducer G proteins in both brain and heart (e.g., Biochem. Pharm. 26:1201, 1987). We studied the influence of halothane on the muscarinic receptor-mediated stimulation of GTP hydrolysis by G proteins in membranes isolated from rat striatum and cerebral cortex. Membranes were incubated with gamma-[^{32}P]GTP for 1-10 min at 37° in a regenerating buffer containing 2 uM GTP. Basal GTPase activity was 56 ± 15 pmol Pi released/mg protein/min in the striatum. ACh stimulated the low K_M GTPase activity by 40-80%, with an EC50 of 2 ± 1 uM and a maximal effect with 10-20 uM ACh. Pretreatment of the membranes with pertussis toxin under ADP-ribosylating conditions decreased both basal and receptor-stimulated GTPase activity by up to 60%. Equilibration of the membrane with halothane (0.1-4%) resulted in a decrease in ACh-stimulated, but not basal, GTPase activity. The IC50 for this action was about 0.5%. These findings suggest that halothane selectively interferes with receptor-G protein coupling. (Supported by GM-37948, AA-07698 and the Georgia Heart Association.)

325.19

ISOFLURANE STRONGLY DEPRESSES POSTSYNAPTIC POTENTIALS IN NEOCORTEX. H. El-Beheiry* and E. Puil. Depts. of Anaesthesiology and Pharmacology & Therapeutics, Univ. of British Columbia, Vancouver, Canada, V6T 1W5.

An anaesthetic enhancement of neuronal inhibition has been suggested as a possible mechanism of anaesthesia in allocortex but has not yet been demonstrated in neocortex. In the present investigations, the effects of a clinical agent, isoflurane, on excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) were investigated intracellularly in neurons of in vitro slice preparations of anterior cingulate and sensorimotor cortex (guinea pig). The microelectrodes contained 3 M KCl, K-acetate, or Cs_2SO_4. The membrane potentials were maintained at a constant resting level (~-65 mv) by DC-current injection. Applications (1-2 MAC) of isoflurane induced a dose dependent, reversible depression of EPSPs and IPSPs evoked by subpial electrical stimulation. Bicuculline (50μM) was applied in the bath to prevent summation of GABA-ergic IPSPs with the EPSPs. With bicuculline-blockade of IPSPs, isoflurane applications (1.5 MAC) markedly depressed the EPSPs. The IPSPs were evoked in either the absence, or presence of Cs^+-blockade of K^+-channels. In the latter case, isoflurane applications (1.5 MAC) reduced the amplitudes of IPSPs by 40%. The attenuation of IPSPs can account for the excitatory phenomena observed clinically during isoflurane administration. These investigations demonstrate that applications of isoflurane depressed the excitabilities of neocortical neurons by interfering with synaptic excitation rather than by potentiating neuronal inhibition. (Supported by the MRC).

326.1

MECHANISM OF ETHANOL POTENTIATION OF GABA-INDUCED $^{36}Cl^-$-INFLUX IN CULTURED SPINAL CORD NEURONS. A.K. Mehta* and M.K. Ticku, Dept. of Pharmacology, Univ. of Tx. Hlth. Sci. Ctr., San Antonio, TX 78284-7764.

$^{36}Cl^-$-influx; while at concentrations ≥ 50 mM ethanol activated Cl^- channels directly. The effect of ethanol was specific for $GABA_A$ receptor-gated Cl^- channels, since ethanol did not potentiate glycine-induced $^{36}Cl^-$-influx in the same neurons. Ethanol (20 mM) which does not exhibit a direct effect, decreased the K_m value of GABA from 11.4 ± 0.8 μM to 4.8 ± 0.5 μM without affecting the maximal response. Both the enhancing and direct effects of ethanol on $^{36}Cl^-$-influx were blocked by GABA antagonists such as bicuculline, picrotoxinin, and inverse agonists of the benzodiazepine site such as RO15-4513 and FG-7142. Ethanol potentiating effect of GABA-induced $^{36}Cl^-$-influx was also reversed by DMCM. The effects of the inverse agonists were blocked by the benzodiazepine receptor antagonist RO15-1788. Both RO15-4513 and FG-7142 reversed direct and GABA potentiating effects of ethanol effect at concentrations lower than those that exhibit inverse agonistic activity in the $^{36}Cl^-$-influx assay in cultured neurons. These results suggest that ethanol facilitation of GABAergic events involves GABA receptor-gated Cl^- channels and that this interaction may be responsible for some of the pharmacological effects of ethanol. Supported by NIAAA grant #AA04090.

326.2

Effects of continuous benzodiazepine antagonist exposure on GABA/Bz ionophore complex. C. Heninger, M. Wilson and D. Gallager, *Dept. of Psychiatry, Yale U. Sch. Med., New Haven, Ct. 06508.*

We have previously demonstrated regionally specific decreases in GABA sensitivity following chronic bz agonist exposure. Based on evidence suggesting the existance of endogenous ligands for the bz binding site, such changes could be due either to direct agonist effects within the GABA/bz complex or to displacement of endogenously active substances at the receptor. We have therefore investigated in rats the effects of chronic exposure to a bz antagonist, Ro15-1788, which would occupy the binding site without inducing agonist effects. Chronic exposure consisted of s.c. silastic implants containing crystalline Ro15-1788 which maintained brain levels of ~20 ng/g throughout the 3 week treatment period. This level of Ro15-1788 produces receptor occupation comparable to that seen with chronic agonist exposure. Following this treatment, we examined the sensitivity of dorsal raphe (DRN) neurons to iontophoretically applied GABA. While we have documented subsensitivity to GABA following comparable chronic agonist exposure, Ro15-1788 treatment failed to alter DRN GABA sensitivity. In addition, GABA/Bz binding parameters (3H-flunitrazepam, 3H-bicuculline) in cortical membranes were unaltered by chronic antagonist exposure. Finally, no significant change in bicuculline seizure threshold was observed after chronic antagonist treatment or after agonist challenge in chronic Ro15-1788 rats as compared to control animals. Such data indicate a lack of effect of chronic antagonist exposure on functional and biochemical measures of the GABA/Bz complex.

326.3

CHRONIC DIAZEPAM ALTERS GABA-STIMULATED CHLORIDE INFLUX IN CORTEX, BUT NOT CEREBELLUM. R.J. Marley and D.W. Gallager, *Dept. of Psychiatry, Yale U. Sch. Med., New Haven, Ct. 06508.*

Clinical and behavioral studies have shown that tolerance develops to many of the therapeutic actions of benzodiazepines, however, the mechanisms responsible for the development of tolerance to benzodiazepines is unknown. Recent studies in our laboratory (Wilson and Gallager, Eur. J. Pharmacol., 136:333, 1987) have found regional differences in the effects of chronic diazepam treatment. Using diazepam-filled silastic implants to maintain pharmacologically relevant brain concentrations of diazepam, it was observed that rats exposed to diazepam for 3 weeks show a decreased responsiveness of dorsal raphe neurons to iontophoretically applied GABA. In the substantia nigra pars reticulata, however, the same treatment failed to alter GABA sensitivity. Using the same sustained release chronic treatment protocol, we have measured GABA-stimulated $^{36}Cl^-$ influx into brain membrane vesicles from rats treated chronically with diazepam or vehicle. To investigate possible regional differences in response to chronic diazepam exposure, we have analyzed vesicles prepared from cortex and cerebellum. Cortical membrane preparations from chronic diazepam-treated rats exhibited a decreased GABAergic stimulation of $^{36}Cl^-$ influx. In contrast, chronic diazepam treatment had no effect on GABA-stimulated $^{36}Cl^-$ influx in cerebellar membrane preparations. These results support the suggestion that chronic benzodiazepine treatment results in a reduction in GABA/BZ receptor function in some, but not all, brain regions.

326.4

GABA SUBSENSITIVITY OF DORSAL RAPHE NEURONS IN MIDBRAIN SLICES FROM CHRONIC DIAZEPAM-TREATED RATS. M. A. Wilson and D. W. Gallager, *Dept. Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508.*

We have previously demonstrated GABA subsensitivity in dorsal raphe neurons (DRN) following chronic BZ agonist exposure. Our prior studies analyzed *in vivo* sensitivity of DRN to iontophoretically applied GABA in anesthetized rats following prolonged exposure to diazepam-filled silastic capsules. To determine whether GABA subsensitivity was dependent upon dorsal raphe afferents, we analyzed GABA responses of DR neurons in brain slices obtained from rats exposed to diazepam-filled silastic capsules for 3 wks. Midbrain slices from vehicle and diazepam-treated rats were maintained simultaneously under the same *in vitro* conditions. Artificial cerebrospinal fluid containing 2.5 uM phenylephrine was used to induce pacemaker-like activity. Firing rates of DRN in control and diazepam-treated slices were comparable (19±1 vs 18±2 spikes/10 sec), indicating that sensitivity to phenylephrine was not altered by chronic diazepam exposure. DR neurons in slices from chronic diazepam-treated rats had reduced responses to iontophoretically applied GABA, when compared to the GABA sensitivity observed in control slices (GABA IT50s: 25±2 in controls vs 34±4 nA.sec in dz-treated). Sensitivity of DRN to iontophoretically applied 5-HT was similar in control and diazepam-treated slices (5-HT IT50s: 691±66 vs 571±75). Thus, following chronic diazepam exposure, subsensitivity to GABA is observed in DR neurons both *in vivo* and *in vitro*. This suggests that the decreased GABAergic responses induced by chronic diazepam exposure reflect changes intrinsic to the dorsal raphe nucleus.

326.5

MODULATION OF GABA-MEDIATED DEPOLARIZING SYNAPTIC RESPONSES BY NMDA IN IMMATURE HIPPOCAMPAL NEURONS R. Corradetti*, J.L. Gaiarsa*, Y. Ben-Ari and E. Cherubini (SPON : R. Adamec), INSERM U-29, 123 Bd Port-Royal, Paris 14, FRANCE.

Intracellular recordings were made from immature CA3 rat hippocampal neurons (0-8 days) in the in vitro slice preparation. In adult CA3 pyramidal neurons, stimulation of the hilus evokes at resting membrane potential (RMP) an EPSP followed by an IPSP. The EPSP is mediated by an excitatory amino acid acting on non-NMDA receptors, whereas the IPSP by GABA acting on GABA A and GABA B receptors. In newborn rats, stimulation of the same region evoked a long-lasting (400 ms) depolarizing potential which was mediated by GABA acting on GABA A receptors since it was blocked by bicuculline (10 uM). The reversal potential of this response (-29 ± 4 mV, n=9 with K Cl- and -53 ± 4 mV, n=8 : mean ± SEM with K-acetate-electrodes) was very close to the reversal of the response to exogenously applied GABA (-30 mV and -59 mV with K Cl- and K -acetate respectively). NMDA receptor antagonists (APV, AP-7 : 50 uM ; CPP : 30 uM) greatly reduced or abolished the evoked responses. In addition, in newborn but not in adult animals, spontaneous giant depolarizing potentials were recorded during the first week of life. These potentials were abolished by bicuculline or APV.

We conclude that, in immature hippocampus, evoked and spontaneous giant synaptic potentials are mediated by GABA which is depolarizing at RMP and whose release is controlled by NMDA receptors. RC is recipient of an EMBO fellowship.

326.6

INHIBITION OF GABA-STIMULATED CHLORIDE INFLUX INTO MEMBRANE VESICLES FROM RAT CEREBRAL CORTEX BY ANTIDEPRESSANTS AND NEUROLEPTICS. M.Ikeda*, E.Malatynska*, R.F.Squires[1] and H.I.Yamamura. Dept. of Pharmacology, Univ.of Arizona, Tucson, AZ 85724 and [1]Nathan Kline Inst.for Psychiatric Res. Orangeburg, NY 10962.

One of the important side-effects produced by clinically used antidepressants and neuroleptics is convulsive seizures. The pharmacological basis of the convulsive effects is not known. To elucidate the convulsive effects of antidepressants and neuroleptics, we examined the effects of amoxapine, SK&F 10810, maprotiline, (-)mianserin, chlorpromazine, haloperidol, pimozide and zotepine on GABA-stimulated $^{36}Cl^-$ uptake into membrane vesicles from rat cerebral cortex. Aliquots of membrane vesicles were preincubated with test compounds for 10 min. $^{36}Cl^-$ uptake was initiated by the addition of $^{36}Cl^-$ and GABA solution to the tubes containing membrane vesicles. After incubation for 3 sec, the uptake was terminated by the addition of ice cold buffer followed by rapid vacuum filtration through Whatman GF/C filters. Amoxapine, maprotiline and (-)mianserin inhibited GABA-stimulated $^{36}Cl^-$uptake at 1uM, 44.1+/-4.8%, 30.3+/-4.9% and 41.0+/-3.8% (% of inhibition), respectively. Some neuroleptics showed inhibition of GABA-stimulated $^{36}Cl^-$ uptake at 10uM and 100uM concentration. These findings suggest that the convulsive effects of some psychotropic drugs are related to the GABA-gated chloride influx.

326.7

ß-CCE, CGS8216 AND Ro15-1788: A COMPARISON OF ACTIVITY IN MOUSE AND RAT. L.S. Labinsky* and J.L. Vaught. Janssen Research Foundation, Spring House, PA 19477

In both mouse and rat, ß-CCE, CGS8216 and Ro15-1788 selectively block the anticonvulsant activity of diazepam (but not phenobarbital) in a metrazol seizure test. However, in the mouse, ß-CCE and CGS8216 are also potent proconvulsants. In the metrazol or electrically-induced seizure threshold tests, ß-CCE at doses <5 mg/kg i.p. and CGS8216 at doses <1 mg/kg i.p. but not Ro15-1788, potentiate convulsions [proconvulsant ED50 in electrically-induced seizures: CGS8216=0.8 (0.4-1.6) mg/kg i.p.; ß-CCE=3.8 (1.8-7.6) mg/kg i.p.]. Their proconvulsant duration of action is <2 h. Thus, in the mouse, CGS8216 and ß-CCE are more potent proconvulsants than they are BZD antagonists. Unlike ß-CCM (CD50~9 mg/kg i.p.) or DMCM (CD50=3.9 mg/kg i.p), neither ß-CCE (>100 mg/kg) nor CGS8216 (>20 mg/kg) causes convulsions. In the rat, however, ß-CCE produces weak proconvulsant activity while CGS8216 is practically devoid of proconvulsant effects in either chemical or electrical-induced convulsions. Thus, in the rat, these agents are more BZD antagonist-like than proconvulsant. These data suggest: 1) as previously reported, Ro15-1788 remains the "purest" of BZD antagonists, 2) that CGS8216 and ß-CCE are similar in their effects on seizure threshold and their relative potency is consistent with binding affinity and 3) that potential differences in the pharmacophysical characteristics of the GABA/BZD complex exist between mouse and rat.

326.8

SKF88901A AND SKF89976A (POTENT GABA UPTAKE INHIBITORS): A COMPARISON OF ANALGESIC AND ANTICONVULSANT ACTIVITY IN MOUSE AND RAT. J.L. Vaught, L.S. Labinsky and P.E. Setler. Janssen Research Foundation, Spring House, PA 19477

SKF89976A [N-(4,4-diphenyl-3-butenyl)-nipecotic acid] and its ethyl ester SKF89901A (Yunger et al., JPET 228:109, 1984) were confirmed to be potent inhibitors of the uptake of GABA in rat brain synaptosomes (K_I values = 0.4 and 10.0 µM, respectively). Administered i.p. to mice, both compounds produced atropine-sensitive, naloxone, picrotoxin and bicuculline insensitive analgesia in the mouse tail flick and hot plate (48°C) tests (ED_{50}'s ranging from 5 to 25 mg/kg). Rotorod performance was not impaired by either compound at doses as high as 50 mg/kg. Tolerance developed to the analgesic effects of SKF89976A following chronic dosing (ED_{50} naive = 13.2 mg/kg ; ED_{50} chronic drug = 148.8 mg/kg i.p. hot plate). In the rat, however, no analgesic activity was observed at doses as high as 100 mg/kg. Both compounds effectively block metrazol-induced convulsions in mouse and rat with ED_{50} values between 1 and 8 mg/kg i.p. Given the similar potency in both species as an anticonvulsant, the marked differences between these species in regard to analgesic activity and the relatively inconsistent analgesic response, we suggest that these GABA-uptake inhibitors show more promise in preclinical tests as anticonvulsants than as analgesics.

326.9

STRUCTURE ACTIVITY RELATIONSHIPS FOR STEROID POTENTIATION OF GABA RECEPTOR-MEDIATED CHLORIDE TRANSPORT IN RAT CEREBRAL CORTEX. A. L. Morrow[1], R. H. Purdy[2]* and S.M. Paul[1]*. [1]Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892, and [2]Southwest Foundation, San Antonio. TX. 78284

Endogenous metabolites of progesterone and deoxycorticosterone have recently been shown to enhance the effect of γ-aminobutyric acid (GABA) on chloride ion flux in spinal cord neurons and in rat brain synaptoneurosomes. The ring-A reduced metabolites, tetrahydrodeoxycorticosterone (THDOC) and 3-α-hydroxy,5-α-dihydroprogesterone (3-α-OH,5-α-DHP) were found to be extremely potent (EC_{50} ~ 120 nM) modulators of muscimol-stimulated $^{36}Cl^-$ uptake *in vitro*. We, therefore, examined a series of related compounds in order to determine the structural requirements for activity on GABA receptor function. Synaptoneurosomes were prepared from cerebral cortices of adult male S.D. rats. Muscimol-stimulated $^{36}Cl^-$ uptake was measured for 5 seconds in the presence or absence of various steroids (1 µM). THDOC potentiated the effect of muscimol (3 µM) by 16.1 +/- 1.5 nmole/mg protein. THDOC-21-acetate had similiar activity increasing muscimol-stimulated $^{36}Cl^-$ uptake by 12.2 +/- 1.1 nmol/mg prot. 3-α-OH,5-α-DHP and 3-α-OH P potentiated muscimol-stimulated Cl^- flux by 10.9 +/- 2 and 10.4 +/- 1 nmol/mg protein respectively, while 3-β-OH P was inactive. Analogs of these active compounds containing unsaturated C rings were also inactive. Other inactive derivatives were 3-α-OH,5-α-DHP, 3 acetate and THDOC, 3 acetate. The glucocorticoids and mineralcorticoids were inactive at concentrations between 0.2 and 5 µM. These data define the specificity for steroid hormone metabolite activity on GABA-mediated chloride ion flux in the rat brain.

326.10

PRETREATMENT WITH ETHANOL POTENTIATES INHIBITION OF GABA-ACTIVATED CHLORIDE FLUX BY BENZODIAZEPINE INVERSE AGONISTS. K.J. Buck*, A.M. Allan, and R.A. Harris. Department of Pharmacology, Univ. Colo. Hlth. Sci. Center, Denver, CO 80262; and VA Med. Res. Serv., Denver, CO 80222.

We found that the actions of benzodiazepine (BZ) inverse agonists on muscimol-activated $^{36}Cl^-$ uptake were enhanced by pretreatment with ethanol. Ethanol pretreatment in vitro or in vivo was effective. Cortical membrane vesicles (microsacs) were prepared from acutely treated mice or microsacs were treated in vitro and then washed. Basal and muscimol-activated $^{36}Cl^-$ uptake were measured as previously described (Life Sci. 39: 2005, 1985). The BZ inverse agonists used were methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidizo[1,5a] [1,4]-benzodiazepine-3-carboxylate (Ro 15-4513) and N-methyl-β-carboline-3-carboxamide (FG7142).

Sensitization to DMCM was the result of a shift in the potency of DMCM, rather than an increase in the maximal inhibition. The actions of DMCM were blocked by the central benzodiazepine receptor antagonist Ro 15-1788 (10 uM).

Our results indicate that pretreatment with ethanol alters sensitivity of the benzodiazepine site to inverse agonists. This sensitization may play an important role in the development of acute tolerance to and withdrawal from ethanol, as well as the antagonism of ethanol intoxication by BZ inverse agonists. Supported by the VA and AA06399.

326.11

ONTOGENY OF GABA-STIMULATED CHLORIDE UPTAKE AND ITS ENHANCEMENT BY DIAZEPAM IN RAT BRAIN SYNAPTONEUROSOMES. C.K. KELLOGG AND G.L. PLEGER*, DEPT. OF PSYCHOLOGY, UNIV. OF ROCHESTER, ROCHESTER, NY 14627.

Late gestational exposure to diazepam (DZ) has been shown to induce selective and lasting alterations in the offspring (Kellogg, Prog. Brain Res., Vol. 73;1988). Ligand binding assays have indicated the presence of DZ at appropriate receptor sites in fetal brain following administration of the drug to the dam. The present study was undertaken to determine whether DZ occupancy of its recognition site in fetal brain could elicit an effector response. In the adult organism DZ facilitates GABA stimulation of chloride (Cl^-) flux. In the present study, the ability of DZ to enhance GABA- stimulated $^{36}Cl^-$ uptake was examined in synaptoneurosomal preparations of whole brain at 20 and 21 days of gestation and of cerebral cortex from 7 to 90 days of age. GABA-stimulated Cl^- uptake was detectable from 20 days of gestation with an increase in EC_{50} for GABA occurring from 20 to 21 days gestation (from 5.1 to 11.3 µM). Maximal stimulation of uptake during this period occurred at 50-100 µM GABA and was 4.76 ng/mg protein/10 sec at 20 days and 6.13 at 21 days. DZ decreased the EC_{50} for GABA stimulation by 28% (a magnitude similar to that observed in adult tissue) without changing the maximal stimulation of Cl^- uptake. Following birth, the EC_{50} for GABA stimulation was similar to adult values (8µM) at 7 and 14 days, but DZ induced a considerably greater shift (50%) in the EC_{50} at these ages than observed in fetal or adult tissue. At 21 days, the EC_{50} for GABA-stimulation increased to 11.25 µM, but the effect of DZ was still pronounced. By 28 days postnatal age, the EC_{50} and the response to DZ appeared adult-like. The maximal stimulation increased throughout development reaching peak values (19 ng/mgprotein/10 sec) by 21 days. These results indicate that DZ is capable of eliciting an effector response by late gestation in the rat fetus. The developmental shifts in the EC_{50} for GABA stimulation of Cl^- uptake and in the responsivity to DZ suggest underlying changes in the polymolecular complex containing the DZ and GABA recognition sites and the Cl^- ionophore. Supported by grant no.MH31850.

326.12

DIBUTYRYL ^{36}cAMP AND FORSKOLIN DECREASE $GABA_A$ RECEPTOR-MEDIATED $^{36}Cl^-$ UPTAKE IN RAT BRAIN SYNAPTONEUROSOMES. Gunter Heuschneider* and Rochelle D. Schwartz. Dept. Pharmacology, Duke Univ. Medical Center, Durham, NC.

The functional activity of the GABA receptor-coupled Cl^- channel was measured under cAMP dependent phosphorylating conditions. Pretreatment of rat cerebral cortical synaptoneurosomes (10 min, 30° C) with dibutyryl cAMP (Bt_2cAMP, 0.1-3.0 mM), produced a concentration-dependent decrease of muscimol-induced $^{36}Cl^-$ uptake (5.2 ± 1.3 - 55.5 ± 2.3%). Bt_2cAMP (1 mM) decreased the maximal effect (25.3 ± 4.0%) but not the potency of muscimol to stimulate $^{36}Cl^-$ uptake. Forskolin (FSK, 30-200 uM) also inhibited muscimol-induced $^{36}Cl^-$ uptake (18.3 ± 1.5 - 42.0 ± 3.9%). The inhibitory effect of FSK (50 uM) was near maximal by 5 sec preincubation. The same time course was observed for FSK-induced cAMP generation in the intact synaptoneurosomes. However, the inactive FSK analog, 1,9-dideoxyforskolin (20-200 uM), similarly inhibited the muscimol response (19.3 ± 4.5 - 67.5 ± 5.1%), indicating the effect of FSK might also involve mechanisms unrelated to activation of adenyl cyclase. The inhibition of the muscimol response by both Bt_2cAMP (1 mM) and FSK (50 uM) was attenuated (16.0 ± 1.2 and 12.2 ± 1.9%, respectively) in Ca^{2+}-free buffer. These data suggest that cAMP-dependent phosphorylation mechanisms regulate the activity of the GABA receptor-gated Cl^- channel in brain.

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326.13

A COMPARISON BETWEEN EFFECTS OF FLURAZEPAM AND Ro07-0213 REVEALES INTRACELLULAR LOCI OF ACTION OF BENZODIAZEPINES IN ADULT RAT NEURONS. J.-I. Oka, E. Sakurai* and H. Fukuda*. Dept. Toxicol. Pharmacol., Fac. Pharma. Sci., Univ. Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

It is assumed that the main effect of benzodiazepines (BZD) is potentiation of γ-aminobutyric acid (GABA) action through their binding sites. However, high lipid solubility of BZD suggests rapid penetration through the plasma membrane and responses induced intracellularly. In this study, we compared the effects of flurazepam (FLU) and Ro07-0213 on dorsal root ganglion cells which were isolated from rats (250-350g) anaesthetized with urethane/α-chloralose. Ro07-0213 is a quaternary derivative of FLU, and would not be expected to pass across the membrane (czajkowski, C. & Farb, D.H., J. Neurosci., 6:2857, 1986). FLU and Ro07-0213 showed similar potentiating effects on GABA-depolarization. Trypsin-treatment (7990u/mL) abolished the GABA action and also the BZD effects, while electrical membrane properties were not affected. These results assure that BZD binding sites related to these effects are exposed at the extracellular surface. Augmentation of the membrane resistance by FLU was long-lasting, whereas that by Ro07-0213 disappeared just after termination of its application. The effects of Ro07-0213 but not FLU were abolished by trypsin. FLU preferably depressed the steady-state outward currents, whereas Ro07-0213 modified an earlier phase of outward currents as well. These results suggest existence of intracellular loci of action of BZD as well as extracellular binding sites.

326.14

GABA DIRECTLY ACTIVATES Cl^- CHANNELS IN ASTROCYTES OF HIPPOCAMPAL SLICES. S.A. Crichton(1), B.A. MacVicar(1), F.W.Y. Tse(1) and H. Kettenmann(2) (1) Neuroscience Research Group, University of Calgary, Calgary, Alberta, T2N 4N1 and (2) Department of Neurobiology, I.M. Neuenheimer Feld 364, 6900 Heidelberg F.R.G.

Glial cells in culture have GABA activated Cl^- channels (Backus et al, Glia in press). We have used kainic acid lesioned hippocampal slices to examine the responses of astrocytes to GABA in a neuron free environment. Intracellular recordings were obtained from astrocytes in the CA3 region of slices from hippocampi which had been lesioned by an i.c.v. kainic acid injection one month previously. Membrane potential of astrocyes averaged -85 mV and neurons were never impaled. GABA perfusion depolarized glial cells (1mM, 1.6 ± 0.7 mV n = 10; 10 mM, 3.5 ± 0.9 mV n = 9) as did a GABA agonist, muscimol (1 mM, 2.7 ± 1.8 mV n = 5). Picrotoxin (10^{-4} M), a Cl^- channel antagonist, reversibly decreased the GABA depolarization by 40% whereas β-alanine, which blocks glial GABA uptake had no effect (n = 3). Pentobarbital (10^{-4} M) and flunitrazepam (10^{-5}) enhanced the GABA response by 60% (n = 9) and 50% (n = 4) respectively. These results indicate that astrocytes have GABA activated Cl^- channels which are modulated in a similar manner to neuronal $GABA_A$ receptors. GABA activated glial Cl^- currents could help regulate the extracellular milieu.

326.15

THE REVERSAL POTENTIAL FOR GABAERGIC CURRENT IS INFLUENCED BY MEMBRANE VOLTAGE. J.A. Hirsch and P.R. MacLeish. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

Recordings made in slices of visual cortex (Scharfman and Sarvey, Neurosci. 23:407-422,1987) show that GABA is hyperpolarizing when applied to cell bodies and depolarizing when applied to distal dendrites. We attempted to study this regional difference in chemosensitivity by applying GABA to the somata and processes of visual cortical neurons maintained in culture. The occipital cortex of embryonic and postnatal rats was dissociated and plated onto a lawn of flat, nonneuronal cells or an antibody to Thy-1. Cultures were maintained for 1 to 6 weeks in L-15CO_2 with 5% rat serum. With standard whole-cell, patch-clamp techniques, recordings were made from cells bathed in a modified Hank's solution warmed to ~34°C. Patch pipettes contained, in mM: K gluconate, 140 mM; EGTA, 1.1; Hepes/KOH 10; $MgCl_2$, 2; NaCl,2; Na_2ATP, 2; Na_2GTP, 0.1; glucose 10. GABA, 50-100 μM, was applied by pressure from pipettes with tip diameters of 2 -4 um positioned near the cell surface. The reversal potential for GABA applied either at the soma or at distances of 50 to 150 um along dendrites was similar and within 5 mV of the holding level when cells were clamped at -58 to -60 mV. When the membrane was depolarized, E_{GABA} began to climb and, in minutes, stabilized near the new holding value. The rate of shift in reversal potential ranged from 6 - 10 mV per 10 mV increment in membrane voltage (n=12). Displacing the holding potential below -60 mV caused smaller changes in E_{GABA}. The effects of altering the membrane voltage were completely reversible. The actions of GABA were blocked by 10 μM picrotoxin and mimicked by 100 μM muscimol but not by 500 uM baclofen. Our results suggest that synaptic transmission involving $GABA_A$ receptors may be regulated by any factor that affects the membrane voltage. If, in tissue, dendrites are maintained at potentials more depolarized than at the soma, the voltage sensitivity of E_{GABA} could account for some of the previously-observed differences in response to the transmitter. Supported by NIH-EY06010,-EY05201,The Klingenstein Fund and a Javits Center Award.

326.16

RESPONSE OF THE PERIAQUEDUCTAL GRAY (PAG) NEURONS TO GABA AND ITS ANTAGONISTS M.M. Behbehani, S.D.Chandler* and M. Ennis. Dept. of Physiology and Biophysics, U. Cincinnati College of Med. Cincinnati,OH 45267-0576.

There is indirect evidence that a tonically active inhibitory GABAergic network within the PAG plays a significant role in activation of the PAG- mediated analgesia. We examined the effect of GABA,picrotoxin (PTX) and bicuculline methiodide (BICM) on baseline activity of PAG cells in PAG slices as well as in anesthetized rats.

Recordings were made from neurons in PAG slices that were perfused with normal or calcium free $CoCl_2$ containing Krebs-Ringers solution (KRS). Drugs were applied next to the recording cell by pressure . In the in vivo experiments, drugs were applied by iontophoresis or by pressure injection to PAG neurons in choloral hydrate anesthetized 250-300 gram rats.

Response of 50 cells in PAG slices and 16 cells in intact animals were examined. Injection of GABA produced inhibition in 87% of PAG cells and its effect could be blocked by BICM and PTX. Sensitization of response to GABA was noted in 60 % of the cells. BICM increased the base line firing rate in 67% of cells in the in vitro experiments. Similar effect was also observed when the tissue was bathed in calcium free- cobalt containing KRS . In the intact preparation, application of BICM increased the base line firing rate in 62% of the cells. Application of BICM caused multiple spiking of PAG cells in both types of preparations. Neurons in all regions of the PAG were responsive to GABA and BICM and no site specificity of their effect was noted. Supported by PHS grant #20643.

326.17

GABA-EVOKED RESPONSES OF MYELINATED DORSAL AND VENTRAL ROOT FIBERS: MODULATION BY K^+ CHANNEL BLOCKERS. S. Liske and M.E. Morris. Department of Pharmacology, University of Toronto, Toronto M5S 1A8.

GABA (γ-aminobutyric acid) and THIP (4,5,6,7-tetrahydroisoxazol-(5,4-c)pyridin-3-ol) evoke multi-component changes in excitability of dorsal (DR) and ventral (VR) root fibers of isolated bullfrog sciatic nerve — reflected by changes in A-fiber 1/2-maximal compound action potential responses. Initial excitability increases evoked in both roots by GABA are enhanced by 3 mM TEA (VR>>DR); later/secondary components are depressed (DR>VR). TEA also decreases THIP-evoked DR depolarization and augments the remarkably brief VR response. 2 mM CsCl, 50 μM 4-AP and 1 mM $BaCl_2$ depress/delay GABA agonist responses. Block of $GABA_A$-evoked gK or voltage-dependent fast K^+ currents of motor fibers could explain enhancement of early VR depolarizations. Depression of the depolarizations evoked in sensory fibers may signify block of slow K^+ currents and their generation of K^+ accumulation, or of a GABA-evoked inward rectifer. Such differential effects as well as data from curve fitting — using hypothetical reconstructs of depolarizing and hyperpolarizing responses of the roots in the presence and absence of bicuculline methiodide — suggest that GABA-evoked depolarizations are mediated by not only the classical $GABA_A$ receptor but at least one additional receptor/mechanism.

(Supported by The Medical Research Council of Canada).

326.18

EFFECTS OF BICUCULLINE METHIODIDE AND PHACLOFEN ON SPONTANEOUS AND EVOKED PSP'S IN RAT HIPPOCAMPAL ROLLERTUBE CULTURES. A.T. Malouf, C.A. Robbins* and P.A. Schwartzkroin. Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195

We have begun to examine the electrophysiological properties of cultured hippocampal slices prepared by the method of Gahwiler (*J. Neurosci. Methods*, 4:329-342, 1981). Cells in these slices exhibited typical properties for CA1 or CA3 hippocampal pyramidal neurons. Most cultures demonstrated a high incidence of small, fast PSP's; EPSP's could trigger action potentials at a rate of 0-5/sec. These cultures also produced spontaneous slow (up to 1 sec. duration) IPSP's. Stimulation of the stratum radiatum in CA1 or the hilus produced an EPSP followed by a biphasic IPSP, suggesting that the Schaffer collaterals and mossy fiber pathways develop the appropriate pattern of innervation. The $GABA_A$ antagonist bicuculline methiodide (BMI) and the putative $GABA_B$ antagonist phaclofen were bath applied to the cultures. BMI (10 uM) produced a reduction in the number of spontaneous PSP's and repetitive burst discharges; stimulation of the afferent fiber pathway produced a long train of action potentials even at previously subthreshold stimulus intensities. Phaclofen (1 mM) did not produce changes in spontaneous fast PSP's but dramatically reduced the amplitude of the slow IPSP following fiber pathway stimulation. No increase in burst activity or number of spikes in response to pathway stimulation was observed. Our data indicate that both $GABA_A$ and $GABA_B$ receptors contribute to the spontaneous and stimulus-evoked synaptic activity of these cultures.

This study was supported by NIH-NINCDS grant #NS15317.

326.19

(-)BACLOFEN HAS A DUAL MECHANISM OF ACTION IN RAT SPINAL DORSAL HORN IN VITRO. C A ALLERTON*, P R BODEN AND R G HILL. Parke-Davis Res Unit, Addenbrookes Hospital Site, Hills Rd, Cambridge, CB2 2QB, UK.

In dorsal root ganglia, the $GABA_B$ agonist (-) baclofen depresses inward calcium currents and reduces neurotransmitter release (Robertson, B and Taylor W R, Br J Pharmac 89:661, 1986) while in hippocampus (Newberry, N R and Nicoll, R A, Nature 308:450, 1984) it activates an outward potassium current. We have examined the action of (-)baclofen on deep dorsal horn neurones by intracellular recording from 400μM slices of 9-16 day old rat spinal cord. On all neurones studies (-)baclofen (100nM-30μM) had a post-synaptic hyperpolarizing action and reduced apparent input resistance. This response was blocked by intracellular Cs^+ and extracellular Ba^{2+} (300μM). In addition, (-)baclofen depressed both spontaneous and electrically evoked epsps in these neurones. This was not affected by intracellular Cs^+ or extracellular Ba^{2+}. Indeed Ba^{2+} itself produced additional synaptic activity which was depressed by both (-)baclofen and 20mM mg^{2+}. (-)Baclofen therefore in spinal dorsal horn both post-synaptically increases potassium conductance and pre-synaptically depresses calcium (barium) currents.

326.20

PHARMACOLOGY OF INHIBITION IN THE GLOMERULAR LAYER OF THE OLFACTORY BULB. W.T. Nickell, M.T. Shipley and H.J. Duncan. Dept. Anat. & Cell Biol., Univ. Cinti. Coll. Med., Cincinnati, OH 45267

Two different central GABA receptors have been identified: $GABA_A$ receptors are activated by muscimol, and blocked by picrotoxin and bicuculline; $GABA_B$ receptors are activated by baclofen, but not muscimol and are not blocked by picrotoxin or bicuculline. A recent autoradiographic study (Bowery, *et al.*, 1987) demonstrated a striking segregation of these receptors in the olfactory bulb of the rat: $GABA_A$ receptors are present in all layers of the bulb; $GABA_B$ receptors are almost exclusively located in the glomerular layer. We have studied the properties of $GABA_A$ and $GABA_B$ synapses in the mammalian olfactory bulb (OB).

Orthodromic responses were recorded with microelectrodes in the glomerular layer of the OB. With electrical stimulation of the olfactory nerve (ON) using double pulses with intervals of 25 to 200 msec, the magnitude of the second response decreases at shorter intervals. Both baclofen and muscimol inhibited the response to both pulses. Inhibition of the second response was not blocked by picrotoxin.

These results suggest that inhibition is mediated by both $GABA_B$ receptors and $GABA_A$ receptors in the glomerular layer. $GABA_A$ receptors mediate classic postsynaptic inhibition; $GABA_B$ receptors may be the source of GABA-mediated presynaptic inhibition. Staining with GAD antisera suggest that there are large and small GABA terminals in the glomerular layer while there are only small terminals in the infra-glomerular layer. These two classes of terminals may be related to $GABA_A$ and $GABA_B$ synaptic sites. (Supported by: NS23348, US Army DAMD 17-86-C-6005, NIH NS 23523.)

CEREBRAL ISCHEMIA III

327.1

GM1 GANGLIOSIDE PROTECTS LOSS OF PLASMA MEMBRANE FUNCTION AFTER CEREBRAL FOCAL ISCHEMIA. C.G. Wakade,V. Bharucha*, S.E. Karpiak & S.P. Mahadik. Div. Neuroscience, NYS Psychiatric Inst., Depts. of Psychiatry, and Biochemistry & Molecular Biophysics, Physicians & Surgeons, Columbia U., New York.

We have reported that ganglioside GM1 treatment protects acute injury processes (ionic imbalance, edema, loss of membrane fatty acids, Na,K-ATPase and Mg2-ATPase) indicative of plasma membrane failure after global ischemia. To understand the molecular mechanism of these protective effects in ischemia we have used a reproducible model of focal ischemia. Ischemia was produced by combined permanent medial cerebral & common carotid artery occlusion [MCAo+CCAo] with 1 hr temporary contralateral CCAo in rat. Levels of Na,K-ATPase, Mg-ATPase and tissue ions (Na+, K+ & Ca2+) in ischemic tissue (primary and peri-infarct cortical areas) were compared with the contralateral side in rats treated with saline or GM1. In the primary infarct area 72 hrs after ischemia levels of Na,K-ATPase & Mg-ATPase were reduced by 45% & 37% respectively in the saline rats, and both by only 15% in GM1 treated rats. In the peri-infarct areas losses in both enzyme levels in saline treated rats were minimal (<15%), with almost no losses in GM1 treated rats. The tissue levels of Na+ & K+ paralleled the loss of Na,K-ATPase in saline treated animals but the levels of Ca2+ increased slowly, reaching a maximum after 72hrs.

327.2

IN VITRO PROTECTION AGAINST CEREBRAL HYPOXIA BY LOCAL ANESTHETICS. C.A. West-Phelan*, L.F. Lucas*, B.M. Rigor and A. Schurr, Department of Anesthesiology, University of Louisville School of Medicine, Louisville, KY 40292,

Numerous studies have been published in recent years on potential antihypoxic drugs and their pharmacology, where many of them employed in vivo models of cerebral ischemia/hypoxia. For faster and less expensive screening of such drugs we have been using the in vitro rat hippocampal slice preparation. In a recent study we demonstrated the depressive effect of lidocaine on synaptic function using this in vitro system. If such depression is the result of metabolic arrest or ion fluxes attenuation, then lidocaine and other similar local anesthetics should exhibit antihypoxic properties.

Rat hippocampal slices were incubated with non-depressive doses of either lidocaine, 2-chloroprocaine or cocaine 60 min prior to their exposure to 15 min hypoxia. The rate of recovery of synaptic function (evoked population spike) following the hypoxic episode was used as an index of hypoxic damage. Slices treated with 0.1 mM of any of the three local anesthetics exhibited a significant increase in the recovery rate of synaptic function from hypoxia as compared to control, untreated slices.

The antihypoxic properties of local anesthetics may stem from their ability to reduce Na^+ influx (and possibly its concomitant Ca^{2+} influx), or from conservation of ATP via metabolic slow down, or both.

327.3

FREE RADICAL SCAVENGERS PROTECT AGAINST PEROXIDATIVE DAMAGE IN THE HIPPOCAMPAL SLICE. T.C. Pellmar, K.L. Neel and M.L. Moss*. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814.

Hydrogen peroxide decreases synaptic efficacy and impairs postsynaptic spike generation (Pellmar, Brain Res. 364:377). Since the damage is likely to be due in part to free radical generation, the free radical scavengers dimethylsulfoxide (DMSO) and thiourea would be expected to afford some protection.

Slices of hippocampus were prepared from brains of euthanized guinea pigs. Stimulating electrodes were positioned in the stratum radiatum to stimulate afferents to CA1 region. Recording electrodes were positioned in the stratum pyramidale and in the stratum radiatum of CA1 to record the population spike and the population synaptic potential, respectively. Input-output curves were generated by varying the stimulus intensity from 0 to 1.0 mA. DMSO or thiourea was applied for 30 min prior to peroxide exposure. The responses in scavenger were compared to the responses after 30 min in 0.005% peroxide plus scavenger.

Thiourea (0.5 mM) afforded significant protection. Higher doses of thiourea were not as effective since they directly decreased the population spike. DMSO (50 mM ≃ 0.36%) had no direct effects but protected postsynaptic spike generation from peroxide damage. Impairment of synaptic efficacy by peroxide was still present in DMSO. The data support a role for free radicals in peroxide damage of neuronal tissue.

327.4

ISCHEMIC TOLERANCE IS INCREASED IN THE CISTERNAL INFUSION MODEL OF RAISED INTRACRANIAL PRESSURE: POSSIBLE ROLE OF THE EXTRACELLULAR MICROENVIRONMENT IN THE PREVENTION OF EXCITOTOXIC DEGENERATION A.-C. Duhaime and D.T. Ross Department of Clinical Neurosciences, Brown University and Department of Neurosurgery, Rhode Island Hospital, Providence, RI 02902.

Elevation of the intracranial pressure above the mean arterial pressure has been shown to temporarily stop cerebral blood flow . In order to study the effects of raised intracranial pressure on the development of cerebral ischemia, a cerebrospinal infusion model was used to elevate intracranial pressure above mean arterial pressure for various intervals.

Male Long Evans rats were anesthetized with chloral hydrate and xylazine, orally intubated, and artificially ventilated with room air. The femoral artery was cannulated for continuous measurement of arterial blood pressure. Two 25-gauge needles were introduced into the cisternae magnae, one for measurement of intracranial pressure and the other for instillation of artificial cerebrospinal fluid. Intracranial pressure was raised above blood pressure for five to thirty minutes by cisternal infusion of artificial CSF at ~1cc/ minute. The animals were allowed to survive for one to seven days and the hippocampal formation was examined for ischemic change in 33 micron Nissl stained sections by light microscopy.

No significant behavioral or histological changes were seen unless the duration of ischemia was between 25 and 30 minutes, even when the animals survived for up to seven days. The cisternal infusion of large volumes (10-50 cc) of artificial CSF may prevent the accumulation of extrracellular potassium and excitotoxic amino acids which occur in ischemia. As a result it appears that pathological and behavioral changes occur only after periods of ischemia significantly longer than those required to produce these changes in vessel occlusion models. Studies are in progress to determines the extracellular concentration of excitatory amino acids during raised intracranial pressure.

327.5

NOREPINEPHRINE IN THE GERBIL HIPPOCAMPUS: EFFECT OF DSP4 TREATMENT ON PYRAMIDAL CELL LOSS AFTER TRANSIENT ISCHEMIA. K. Nishino*, J. K. Morse, C.-S. Lin, and J. N. Davis (SPON: F. Eldridge). V.A. Medical Center and Duke University Medical Center, Durham, N.C. 27705.

Hippocampal pyramidal cells are vulnerable to transient periods of ischemia in the Mongolian Gerbil and the rat. We measured the levels of norepinephrine (NE), the distribution of tyrosine hydroxylase-immunoreactive fibers and the effect of DSP4 pretreatment on pyramidal cell counts in ischemic Gerbils. The NE levels of were similar in the dentate gyrus and the septal, midlle and temporal portions of the hippocmapal gyrus. Dopamine was barely detectable. The laminar distribution of fibers was similar to the rat, but less fibers were present in the dentate gyrus and more were seen in stratum oriens of CA_1. Animals were pretreated with 100 mg/kg. of DSP4 14 days before undergoing 5 minutes of bilateral carotid artery occlusion and sacrificed 7 days later. There was significant worsening of pyramidal cell loss in the CA_1, CA_3, and CA_4 hippocampal regions of the DSP4-pretreated animals compared to saline-pretreated ischemic controls and unoperated animals (surviving neurons were 11 *vs.* 9%, 71 *vs.* 49%, and 80 *vs.* 66%, respectively; repeated measures ANOVA, $p<0.0001$). Cortical biopsies of the treated animals before sacrifice for cell counting showed that DSP4 treatment had lowered cortical NE (Saline *vs.* DSP4, 0.29 ±0.02, 0.10 ±0.03; $p<0.0001$, "t" test). Our data are consistent with previous reports in rats suggesting that NE neurons may modulate neuronal death in hippocampal pyramidal cells.

(Supported by the VA and NS 06233)

327.6

PHENYTOIN DECREASES THE VOLUME OF ISCHEMIC DAMAGE CAUSED BY MIDDLE CEREBRAL ARTERY OCCLUSION IN F-344 RATS. J.J. Cordon*, P.A. Boxer and F.W. Marcoux, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

The anticonvulsant phenytoin has been reported to be beneficial in a number of models of global cerebral ischemia. The current experiments were designed to determine if phenytoin was also effective in reducing the damage resulting from focal ischemia. The left common carotid artery was ligated along with the ipsilateral middle cerebral artery 3 mm from its origin in F-344 rats. Phenytoin or commercially available vehicle was administered either IP or IV (via a femoral catheter) 30 min and 24 hrs post occlusion. The volume of ischemic damage was assessed morphometrically based upon extravasation of the dye Evans Blue. Phenytoin, 100 mg/kg IP or 30 mg/kg IV, significantly reduced the volume of ischemic damage by 51% and 43% respectively. When given as a single IV dose (30 mg/kg) either 30 min, 2 hr, or 24 hrs post occlusion there was no significant reduction in infarct size, although there was a 30% reduction in the 30 min group. These doses of phenytoin are approximately ten times greater than the anticonvulsant ED_{50} and produce marked ataxia. Phenytoin has been shown to modulate both sodium and calcium flux and this may account for its neuroprotective activity.

327.7

NEW PHARMACOLOGIC APPROACHES TO PROTECT THE RAT BRAIN FROM ISCHEMIA. T. Tominaga, H. Katagi*, M. Katsuoka* and S.T. Ohnishi. Membrane Res. Inst., Phila., PA 19104.

Protective effects of two new drugs against ischemic brain injury were examined using a rat focal ischemia model. They are charybdotoxin (CTX; purified from a scorpion venom), which is a specific inhibitor of the Ca^{2+}-activated potassium channel, and an oligomeric derivative of prostaglandin E_1 (MR-356).

The focal ischemic lesion was produced by permanent ligations of the middle cerebral artery and the common carotid artery (CCA) combined with temporary occlusion (for 60 min) of the contralateral CCA. CTX (0.15 mg/kg) or MR-356 (6 mg/kg) was administered 30 min before or 50 min after the induction of ischemic insult. Three days after the focal ischemia, the formation of brain edema and the changes in ion contents (Na, K and Ca) were measured. Motor deficits and memory disturbance were also evaluated by motor performance tests (which included inclined plane, balance beam and prehensile tests) and the passive avoidance test, respectively.

The brain edema and ionic derangements were reduced in the pre-ischemic treatment with CTX or in the post-ischemic treatment with MR-356. Motor deficits and memory disturbance were ameliorated in accordance with the reduction of brain edema. Possible protecting mechanisms of these new drugs in brain ischemia will be discussed.

327.8

ANOXIC HIPPOCAMPAL DAMAGE IN PRIMARY CULTURES: PROTECTIVE EFFECTS OF GLUCOSE ARE pH-DEPENDENT. G.C. Tombaugh* and R. M. Sapolsky (SPON: J.D. Barchas). Dept. of Biol. Sciences, Stanford Univ., Stanford, CA 94305.

In vivo, hyperglycemia prior to hypoxia/ischemia increases neuronal injury, probably via anaerobic lactic acid production. We have used primary neuronal cultures to study whether the same effect occurs in vitro and to examine the relationships among glucose, pH_o, and anoxia-induced cell death. Hippocampal neurons taken from 18d rat fetuses were maintained at 37°C/10% CO_2 for 14 days. Cells were made anoxic (100% N_2) for 6hr at 37°C and then refed with fresh media containing 5mM glucose. 24hr later, cell viability was measured by lactate dehydrogenase assay. Since anaerobic cell activity was unable to shift media pH significantly, experimental media were adjusted to defined pH values prior to anoxia. At pH 7.4, anoxia in the absence of glucose caused severe damage; glucose concentrations ranging from 1-30mM not only did not worsen damage, but completely prevented cell death. At pH values as low as 6.5, 5mM glucose during anoxia also prevented cell death, but at $pH_o \leq 6.0$ severe damage occurred which was independent of glucose availability. Thus, in this system an effect emerges that is somewhat different from that observed in vivo: glucose exerts a protective influence on hippocampal neurons during anoxia when pH_o is greater than that which autonomously causes cell damage. (Supported by NIH AG-06633).

327.9

(S)-EMOPAMIL: A NEW CALCIUM ANTAGONIST OF THE VERAPAMIL GROUP WITH HIGH CEREBRAL AVAILABILITY AND ANTIISCHEMIC PROPERTIES. L. Szabo*, H.P. Hofmann*, M. Raschack*, L. Unger* (SPON: R. A. O'Brien). Dept. of Neuropharmacology, Knoll AG, D-6700 Ludwigshafen, Federal Republic of Germany

Experimental studies have yielded conflicting results concerning the therapeutic effect of calcium antagonists in different models. Part of the negative findings may be related to the fact that several compounds with well-documented efficacy on the heart or peripheral vessels are unable to reach the brain in sufficient quantities.

(S)-emopamil [(2S)-2-isopropyl-5-(methylphenethylamino)-2-phenylvaleronitrile-hydrochloride] is a recently developed verapamil derivative that crosses the blood-brain barrier easily. Based on measurements of radioactivity after intravenous application of 14C-labeled substances in rats, the relative cerebral concentration of (S)-emopamil was 16 to 18 times higher than that of verapamil. (S)-emopamil was found to prolong the survival of mice in a dose-dependent fashion both during hypoxic hypoxia and global cerebral ischemia whereas verapamil showed only marginal effects.

Apart from its calcium antagonistic properties, (S)-emopamil has exhibited very high affinity to the 5-HT2 receptor in 3H-ketanserin binding test (Ki=4.4 nmol/L). In measurements on rat aortic strips (S)-emopamil's serotonin antagonistic potency was shown to be 10 times as high as verapamil's (EC50: 4.5 and 42 nmol/L, respectively).

Cerebral vascular and metabolic effects of (S)-emopamil were investigated autoradiographically in the rat. Under physiological conditions (S)-emopamil increased cerebral blood flow by up to 100% of control without changing the metabolic rate of glucose. In reversible forebrain ischemia models (S)-emopamil ameliorated postischemic cerebral hypoperfusion, accelerated energetic restitution and reduced delayed hippocampal neuronal necrosis.

These results suggest that (S)-emopamil may be a useful agent for the treatment of cerebrovascular disorders.

327.10

MONOSIALOGANGLIOSIDE EFFECTS FOLLOWING TRANSITORY GLOBAL CEREBRAL ISCHEMIA IN RODENTS. A. Leon, M.S. Seren*, R. Rubini*, A. Lazzaro*, R. Zanoni*, N. Schiavo*, C. Aldinio*, M. Fiori and G. Toffano (SPON: S. Mazzari). Fidia Research Laboratories, Abano Terme, Italy.

Monosialoganglioside (GM1) treatment of animals has consistently been shown to ameliorate outcome following a variety of CNS injuries, including cerebral ischemia. We here report the effects of the inner ester derivative of the ganglioside following transitory 4 vessel occlusion in adult rats (Pulsinelli, W.A. and Brierley, J.B., Stroke 10:267, 1979). The ganglioside was systemically administered at a dose of 20 mg/kg and its efficacy was evaluated by i) monitoring of the cortical EEG and ii) assessment of the degree of morphological damage in the CA1 area of the hippocampus. Preliminary data indicate that the ganglioside improves EEG recovery during the first 2 weeks following the ischemic episode. In addition, the ganglioside increases the percentage of animals showing less severe hippocampal CA1 neuronal loss.

These effects may perhaps be related to the ganglioside prevention of glutamate neurotoxicity observed in vitro (Favaron, M. et al., FASEB J., 2:A824, 1988; Skaper, S.D. et al., this meeting). To further validate such a hypothesis, the ganglioside capability to decrease post-ischemic selective (glutamate-related) neuronal loss is currently being evaluated in gerbils following transitory bilateral common carotid artery occlusion.

327.11

MK 801 MODULATES METABOLIC RESPONSE TO PERINATAL ASPHYXIA. L.R. Ment*, W.B.Stewart, O.A.C.Petroff* and C.C.Duncan*. Depts. Pediatr., Neurol., Surg., & Anat., Yale Univ. Sch. of Med., New Haven, CT 06510.

The N-methyl-D-aspartate (NMDA) receptor antagonist MK 801 (Merck, Sharp & Dohme) prevents neuronal degeneration following ischemia. We hypothesized that blocking the NMDA receptor would prevent the depression of cerebral high energy phosphates produced by asphyxia, & thus prevent neuropathologic damage. Newborn beagle pups (2-7 d) were anesthetized, tracheotomized, ventilated & randomized to asphyxial insult (I = discontinuation of ventilatory support for 5 min) or control (NI) & drug treatment with MK 801 (10 mg/kg i.v.) or saline (S). Pups were pretreated with MK 801 or S, 30 min prior to I/NI. After 5 min of I/NI, brain extracts were prepared for ^{1}H NMR spectral analysis of high energy phosphorylated compounds & lactate levels. MK 801 had no effect on these measures in the NI brains. Phosphocreatine levels were 1.6±0.2, 0.1±0.1, & 0.87±0.03 mmole/kg (mean ± S.D.) for the S/NI, S/I & MK 801/I pups. Cerebral glucose was 1.55±0.07, 1.20±0.07, & 1.78±0.23 for the S/NI, S/I, & MK 801/I, & lactate was 1.16±0.49, 3.94±0.06, & 2.03±0.39. The pH fell 0.9 units for the S/I animals compared to 0.4 units for the MK 801/I pups. Pretreatment with the NDMA receptor antagonist MK 801 in part protects the developing brain against severe metabolic insult & may diminish the neurologic consequences. (Sup. by NS 21946 to LRM)

327.12

EFFECTS OF LY178002 AND LY256548 ON ISCHEMIA-INDUCED BRAIN DAMAGE. J. A. Clemens, M. L. Phillips*, M. E. Roush* and J. A. Panetta*. The Lilly Research Labs, Lilly Corporate Center, Indianapolis, IN 46285.

LY178002 (5-[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methylene-4-thiazolidinone) and its N-methyl analog, LY256548, inhibit the activity of phospholipase A_2,5'-lipoxygenase, and fatty acid cyclooxygenase. Because of these properties we decided to determine their effects on ischemia-induced brain damage. In the first experiment, a 4-vessel occlusion model of global ischemia was used. Adult, male Wistar rats were prepared for experimentation under anesthesia the day prior to compound treatment by permanent cautery of the vertebral arteries and placement of atraumatic clasps around both common carotids. Global ischemia was induced the following day by tightening the clasps for a 30 min period during which the rats became unconscious. Compounds were administered orally at a dose range of 50-500 mg/kg suspended in 2% acacia. Brains were removed 72 hrs after ischemia, sectioned, and evaluated histologically. LY178002 and LY256548 significantly reduced striatal and hippocampal CA_1 damage when given either before or 15 min after the period of ischemia. In the second experiment, oral administration of 200 mg/kg of LY256548 reduced hippocampal CA_1 damage in Mongolian gerbils subjected to 10 min of bilateral carotid occlusion. We conclude from the results of these studies that LY178002 and LY256548 may be useful in protecting against ischemia-induced damage.

327.13

DELAYED TREATMENT WITH THE NMDA-ANTAGONISTS DEXTROMETHORPHAN AND DEXTRORPHAN, REDUCES CEREBRAL DAMAGE AFTER TRANSIENT FOCAL ISCHEMIA. G.K. Steinberg*, J. Saleh, R. DeLaPaz*, D. Kunis and S.R. Zarnegar*. Division of Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305.

Dextromethorphan (DM), a clinically tested antitussive medication and its major metabolite dextrorphan (DX), are N-methyl-D-aspartate (NMDA) antagonists. Both drugs attenuate hypoxic neuronal injury in culture (Goldberg, M.K., et al, Neurosci. Lett. 80:11, 1987) and we have shown that pretreatment with these agents protects against cerebral damage in a rabbit model of focal ischemia (George, C.P., et al, Brain Res. 440:375, 1988; Saleh, J., et al, Soc. Neurosci. Abstr., 1988). We tested the efficacy of post-ischemia treatment with DM and DX in our rabbit model.

Twenty-two male rabbits were anesthetized and underwent transorbital clip occlusion of the left internal carotid artery and anterior cerebral artery for one hour, followed by 4 1/2 hours of reperfusion. One hour after the onset of arterial occlusion, immediately after removing the clips, animals were blindly treated intravenously with 20 mg/kg loading dose, followed by 10 mg/kg/hr of DM (n = 7), 15 mg/kg loading dose, followed by 15 mg/kg/hr of DX (n = 7), or an equivalent volume of normal saline (NS) (n = 8). The formalin-fixed brains were analyzed blindly with magnetic resonance imaging (MRI) using coronal T2-weighted images (TR2500, TE100). Ischemic neuronal damage (IND) was assessed blindly on standard, coronal hematoxylin and eosin sections.

The area of neocortical IND was significantly reduced in the DM group (4.2%, p<.05) and DX group (6.1%, p<.05), compared with the NS controls (36.2%). MRI demonstrated significantly smaller areas of cortical edema in the DM (14.6%, p<.05) and DX (8.0%, p<.01) treated rabbits, compared with the controls (32.9%).

The present study suggests that systemic treatment with DM and DX one hour after the onset of transient focal ischemia can substantially reduce the extent of neocortical neuronal damage and protect against the development of ischemic edema in a rabbit model. These drugs may have therapeutic potential in clinical stroke.

Supported by NIH grant RR05353

327.14

PRETREATMENT WITH THE NMDA-ANTAGONIST DEXTRORPHAN ATTENUATES NEURONAL DAMAGE AND EDEMA IN FOCAL CEREBRAL ISCHEMIA J. Saleh, G.K. Steinberg*, R. DeLaPaz*, D. Kunis and S.R. Zarnegar* Division of Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305

Dextromethorphan (DM), an N-methyl-D-aspartate (NMDA) antagonist has previously been reported to attenuate ischemic neuronal damage when administered prior to the onset of focal cerebral ischemia (George C. et al, Brain Res 440:375, 1988; Prince D. & Feeser H., Neurosci Lett 85:291, 1988). DM is rapidly O-demethylated by the liver to its major metabolite, dextrorphan (DX). DX is also an NMDA antagonist and protects neurons against hypoxic damage in culture (Goldberg, M.P. et al Neurosci Lett 80:11, 1987). We studied the efficacy of systemic pretreatment with DX in our rabbit model of transient focal cerebral ischemia.

Fourteen New Zealand male rabbits were randomized into two equal groups receiving blindly either 24 mg/kg/0.5 hr loading dose, followed by 12 mg/kg/hr intravenous DX or an equivalent volume of normal saline (NS). One and one-half hours after starting the drug, the left internal carotid and left anterior cerebral arteries were transiently occluded for one hour. After 4 hours of reperfusion, the animals were sacrificed and the brains perfused with 10% buffered formalin. The brains were analyzed blindly using magnetic resonance imaging (MRI) with T2-weighted images (TR2500, TE100). Areas of increased signal representing edema were expressed as a percentage of the total cortex. Histological measurements of ischemic neuronal damage (IND) in the cortex were performed blindly at two coronal levels on hematoxylin and eosin stained sections. The total area of IND was calculated as a percentage of total cortex.

DX treated rabbits had significantly less cortical IND (7.4%), compared with the NS group (31.5%; p = .02). The DX treated group also showed a significant decrease in ischemic edema on MRI (21.6%), as compared with the control group (46.3%; p = .03).

Our findings demonstrate the protective role of DX against ischemic neuronal damage and edema when administered before the onset of transient focal ischemia. We recommend DX be considered for use in clinical trials of stroke treatment.

Supported by NIH grant RR05353

ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS

328.1

CORTICAL AND MIDBRAIN CONNECTIONS OF THE INTERMEDIODORSAL NUCLEUS IN THE RAT. T. W. Deacon. Biological Anthropology, Harvard University, Cambridge, MA 02138.

The nucleus mediodorsalis (MD) is the principle thalamic source of projections terminating in prefrontal cortex. Subnuclei within MD have been shown to project to discrete subregions of prefrontal cortex in monkey, cat and rat and to receive afferents from distinct midbrain areas. However, the afferent and efferent connections of the intermediodorsal nucleus (IMD) on the midline between the MD nuclei has not been characterized and distinguished from those of MD. Injections of WGA-HRP by micropipet were centered in IMD in a series of rat brains and the tissue was processed with TMB. In a number of cases cortex and hippocampus overlying the thalamus along the trajectory of the injection were removed by cautery to prevent uptake of tracer that might confound IMD labeled connections. Injections of tracer were also made in midbrain, MD, and cortical structures to verify IMD connections and distinguish them from connections of adjacent areas. After IMD injections labeled cells are observed bilaterally in the central gray area (CGA), raphe nuclei (RN), deep mesencephalic reticular nucleus (DpMe), intermediate gray layer of the superior colliculus (IntG) and substantia nigra (SN). Injections into DpMe or IntG, not overlapping CGA fail to label axons in IMD, but label axons in subdivisions of MD. Injections into MD that do not overlap IMD fail to label CGA. Injections centered in the dorsal or lateral part of CGA label axons in IMD and cells in PV just dorsal to it. Caudal IMD injections produce significant axonal labeling within CGA, while more rostral IMD injections do not. After IMD injection, cingulate, infralimbic, orbital, and insular areas exhibit labeled cells in layer 6 bilaterally. In infralimbic and orbital areas axonal labeling in layers 3 and 1 is also visible. Injections into these cortical areas label cells and axons in IMD. These data suggest that IMD may be a major route through which the central gray area influences prefrontal areas.

328.2

DISTRIBUTION AND MORPHOLOGY OF GABA AND GLUTAMATE IMMUNOREACTIVE NEURONS IN THE THALAMIC MEDIODORSAL NUCLEUS OF THE MONKEY. A. S. Clark, M. L. Schwartz and P.S. Goldman-Rakic. Sect. Neuroanat., Yale Univ. Sch. Med., New Haven CT 06510.

The mediodorsal nucleus of the thalamus (MD) is the principle thalamic relay to the prefrontal cortex. Using GABA(Immunonuclear) and glutamate (gamma-Glu-Glu antisera, provided by J.E. Madl), the present study examined the organization and morphology of GABA and glutamate immunopositive neurons in the adult primate MD, with the aim of providing information on the local circuit neurons and projection neurons of this nucleus.

GABA and glutamate-like neurons were found to differ on several features of their distribution and morphology. GABA positive cells were generally round or ovoid in shape, and typically had soma diameters of 10 μ. In contrast, glutamate lablelled neurons were generally larger (13 μ in diameter), and had more irregularly shaped soma. GABA-positive neurons were organized in ring-like structures, surrounding areas containing few, or no, labelled cells while glutamate immunoreactive cells were more irregularly distributed, often showing a tendency to aggregate in loose clusters.

Because the magnocellular (MDmc) and parvocellular (MDpc) subdivisions of the MD have anatomically and functionally unique cortical targets, we further examined GABA neurons of these two subdivisions to determine if these differences are also evident in their local circuit organization. GABA-positive cells in the MDmc were larger than those in MDpc and the intensity of reactivity was greater in MDmc. Comparison of the densities of GABA-immunoreactive cells revealed not only a greater density of cells in the MDpc, but also that the proportion of all cells which were GABA-positive was greater in the MDpc. These differences in the density and morphology of inhibitory local circuit neurons may contribute to the functional duality of areas of frontal cortex related to these thalamo-cortical pathways. Supported by NS 08019, MH 38546 and NS 22807.

328.3

DIFFERENCES IN THE ORGANIZATION OF GRANULAR FRONTAL ASSOCIATION CORTEX OF PROSIMIAN AND ANTHROPOID PRIMATES. Todd M. Preuss and P.S. Goldman-Rakic. Sec. Neuroanatomy, Yale Sch. of Med., New Haven, CT 06510.

The connections of granular frontal cortex in the prosimian primate *Galago crassicaudatus* were investigated by injecting WGA-HRP or [3H] amino acids in frontal and posterior association cortex of 15 hemispheres. Cyto- and myeloarchitecture (using the Gallyas myelin method) were also examined. Galago organization was compared to that of macaque monkeys (anthropoid primates).

Galagos and macaques share many features of granular frontal organization, including strong connections with posterior parietal cortex. Macaques have two tiers of parietal-recipient areas, an arcuate (Walker's areas 8b, 8a, 45) and a periprincipal ("dorsolateral") tier (9, 46, 12). Galagos possess arcuate-like cortex (Preuss & Goldman-Rakic, Soc. Neurosci. Abtr. 12: 1440, '86), but lack features indicative of periprincipal areas: (1) Individual posterior parietal areas have fewer zones of termination in galago granular frontal cortex than in macaques. (2) The AV and AM thalamic nuclei, which project to principalis cortex, are weakly labeled in galagos (light label in AM, little or none in AV). (3) Galago parietal-recipient cortex does not project densely to the deep tectum or central grey, structures which receive strong projections from macaque dorsolateral areas. (4) The insula projects only lightly to galago granular frontal cortex; macaque areas 46 and 12 have strong insular connections. (5) Several distinctively myelinated principalis zones, e.g., the very light cortex of the ventral bank of the principal sulcus (area 46), are absent in galagos. We interpret these differences as evidence that prosimian primates (such as *Galago*) are lacking some or all of the dorsolateral areas found in anthropoid ("higher") primates and that these areas are evolutionary specializations of anthropoids. Our findings suggest there are qualitative differences among primates in "prefrontal" organization.

328.4

PREFRONTAL CONNECTIONS OF THE PRINCIPAL SULCUS IN THE RHESUS MONKEY. J.F. Bates and P.S. Goldman-Rakic. Sect. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

The principal sulcus of the macaque prefrontal cortex is topographically connected with numerous nonprefrontal cortical areas, including premotor, temporal, and parietal cortices. However, with the exception of the frontal eyefields (Barbas & Mesulam, 1981), the connections between the principal sulcus and other subdivisions of the prefrontal cortex have not been described in detail. We have made small injections of WGA-HRP into seven distinct cytoarchitectonic areas, as defined by Walker (1940), to systematically investigate these prefrontal interconnections.

Our findings indicate that each subdivision of prefrontal cortex examined has reciprocal, columnar connections with the principal sulcus. WGA-HRP labeled cells and terminals were distributed throughout the sulcus but tended to aggregate in two focal areas, the ventral rim of the posterior principal sulcus and a portion of the dorsal rim in the anterior half of the sulcus. Areas 8a and 9 were heavily interconnected with both banks along the entire extent of the principal sulcus, whereas areas 10, 11, 25, and 45 were connected primarily or exclusively with the lower bank. Extensive interconnections between prefrontal subdivisions and the principal sulcus provide a basis for integration of different subsystems of prefrontal cortex. (Supported by MH 38456).

328.5

ORIGINS OF AFFERENTS TO PREFRONTAL CORTEX IN THE RAT. F.Condé, E.Maire-Lepoivre, E.Audinat and F.Crépel (SPON:J-P.Vedel). Lab.Neurobiologie et Neuropharmacologie du Développement, Université Paris-XI, 91405-Orsay, France.

In order to better understand the role of the rat's prefrontal cortex (PFC), retrograde transport of fluorescent tracers was used to define the distribution of afferent neurons from the whole brain. Diamidino yellow and/or true blue were injected (0.1-0.3 ul) into one or two areas of the PFC, ipsi- or contralaterally. After 4 day of survival, perfusion and histological processing were done according to Audinat, Condé & Crépel (Exp.Brain Res. 69:439-443, 1988). Different PFC areas received projections from different parts of the n.mediodorsalis of the thalamus (MD) according to Krettek & Price (J.Comp.Neurol. 171:157-192,1977), although areas of overlap were seen, eg: neurons in centralis MD projected to both insular and prelimbic area (PL). Moreover a band-like organization of thalamo-cortical relations was evident. Areas in PFC could be distinguished on the basis of their afferents: CA1 field of hippocampus and n. paratenialis (ipsi) project massively only to PL whereas N.gelatinosus and primary olfactif cortex project to insular cortex. These results support the hypothesis that the role of insular cortex is to integrate multisensory information while that of medial PFC is to analyze information prior to motor acts.

328.6

SOME OBSERVATIONS OF THE DIFFERENTIAL THALAMIC INNERVATION OF THE RODENT LATERAL AGRANULAR AND INFRALIMBIC CORTICES. L. J. Freedman and M.D. Cassell, (SPON: Dr. Adel Afifi), Department of Anatomy, University of Iowa, Iowa City, IA 52240.

Previous studies have shown that the rat ventromedial (VM) thalamic nucleus sends projection to both the infralimbic (IL) and lateral agranular (AG_L) cortices. To determine whether these projections involve axon collateralization, we conducted a retrograde double labeling experiment with bisbenzimide and fast blue injected in these cortical areas.

Injections in AG_L strongly labeled neurons in the ventroposterolateral (VPL) nucleus, posterior nuclear group, and VM. Somewhat more lightly labeled were neurons in the zona incerta (ZI) and rhomboid nuclei (Rh). Additionally, cells in the nucleus reuniens were very sparsely labeled. Injections in IL labeled neurons in the Re, Rh, and paraventricular nuclei, and usually (but not always) neurons in the medial mediodorsal nucleus and ZI. Neurons in the ventromedial nucleus were never labeled by infralimbic injections. Double labeled neurons were observed very rarely in the Re and Rh.

The lack of labelling in VM neurons following IL injections was particularly surprising. The discrepancy between our results and Herkenham's ('78) anterograde study may be due to a lateral "tail" of the Re we observed just ventral to VM; uptake of tracer by these cells probably explains the labelling seen in IL in previous studies.

The lack of collateralization among thalamic neurons innervating the infralimbic and lateral agranular cortex suggests that the modulation by the thalamus of these two cortical areas is largely separate. The limited collateralization observed in Re and Rh is consistent with the idea (Jones '85) that these nuclei are involved in diffuse cortical activation.

328.7

FUNCTIONAL HETEROGENEITY OF THE RAT PREFRONTAL CORTEX (PFC). M. Rasmussen*, C.A. Barnes, and B.L. McNaughton (SPON: M. W. Dubin). Behav. Neurosci. Program, Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

The nature of spatial deficits found after prefrontal lesions in the rat has been obscured by the differences in procedures used across studies. Kolb, Sutherland & Whishaw (1983) found deficits on the Morris water task, which requires the use of allocentric spatial maps, after large aspiration lesions to the PFC. After, small electrolytic lesions to PFC, Rasmussen, Barnes and McNaughton (1986; 1987) found no impairment on an allocentric short-latency alternation task and only a mild impairment on the radial maze working memory problem. Egocentric short-latency alternation tasks revealed severe deficits with these lesions.

As part of a study used to assess acquisition of three tasks: reference memory (RM), working memory (WM) and short-latency alternation (SLA),we report now on the results of a comparison between two types of lesion to the PFC on the allocentric spatial tasks. In the RM and SLA tasks strategy was controlled (only allocentric solutions were useful). The WM task could be solved by a combination of strategies.

Neither PFC lesion group was impaired on the RM task; both were impaired on the WM task; and only the aspiration group was impaired on the SLA task. The results suggest that small lesions to the PFC do not disrupt allocentric maps. The mild deficit on the WM task can be attributed to impaired egocentric responding.

Since large aspiration lesions to PFC result in impairment on the WM and SLA tasks, but no deficit on the RM task, the data are consistent with a prefrontal disconnection hypothesis similar to that suggested by Gaffan (1983). i.e., the mapping functions of the hippocampus are no longer available to the complex motor programming areas of the basal ganglia. It is suggested that this disconnection becomes critical when task complexity increases and/or the memory function cannot be supplied by other brain areas which remain connected to basal ganglia.

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328.8

SPATIALLY SELECTIVE DISCHARGE OF VISION AND MOVEMENT MODULATED POSTERIOR PARIETAL NEURONS IN THE RAT. L. L. Chen and B. L. McNaughton. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309

Functional properties of neurons of the posterior parietal cortex (Krieg, 1946, J. Comp. Neurol., 84, 221; Kolb et al., 1987, Brain Res. Rev., 23, 127) in freely moving rats were studied in a spatial working memory task on a radial arm maze, using the stereotrode recording technique (McNaughton, et al., 1983, J. Neurosci. Meth. 8, 391). Most of the 145 cells studied exhibited selectivity for a particular state of motion (e.g., left-turns, right-turns, straight running, still). Visual response characteristics were tested by manipulating the room light source. 24% (21/88) of neurons tested were visually responsive, exhibiting either phasic excitation or inhibition with changes in room illumination or preferentially discharging while the animal was at a particular orientation relative to the light source. Some visually responsive cells showed location selective activity reminiscent of hippocampal "place" cells (O'Keefe,1979, Prog. Neurobiol., 13, 419). At least part of this location specificity appeared to be generated by an interaction between sensorimotor and visual inputs. A few cells maintained a spatial firing bias when the light source was extinguished but showed a shift in bias corresponding to shifts in the position of the visible light source. We conclude that some cells in the posterior parietal cortex could be involved in spatial representation.

(Supported by NS20331 to BLM)

328.9

DISTRIBUTION OF CAT-301 IMMUNOREACTIVITY IN THE POSTERIOR PARIETAL, PREMOTOR AND PREFRONTAL CORTEX OF THE MACAQUE. P.K. McGuire*, S. Hockfield and P.S. Goldman-Rakic, Sect. of Neuroanatomy, Yale Univ. Sch. Med., 333 Cedar St., New Haven, CT 06510.

Monoclonal antibody Cat-301 recognizes a surface antigen on certain C.N.S neurons. It appears to label interconnected areas in the thalamus and cerebral cortex (Hendry et al, J.Neurosci. 8:518 1988), and in the magnocellular visual pathway (DeYoe et al, Soc. Neurosci. Abstr. 12:130, 1986). We have examined the distribution of Cat-301-immunoreactive neurons in regions of the primate posterior parietal and frontal cortex which are strongly and topographically interconnected.

In both, the distribution is characteristic for each cytoarchitectural area, but varies greatly between areas, with abrupt changes at their borders. In posterior parietal cortex (PPC), the most intense immunoreactivity (IR) is in the superior parietal lobule (PE), and the dorsal bank (PEa), fundus (IPd) and inner ventral bank (POa(i)) of the intraparietal sulcus. In frontal cortex (rostral to area 4), labeling is strongest in the supplementary motor and premotor cortex (area 6), and in the caudal prefrontal areas (8a, 8b and 45). Two patterns of neuronal distribution are evident: throughout PPC, and in the heavily-labeled frontal areas, immunopositive cells are concentrated in lower layer III and in layer V, and approximately 15-25% are pyramidal, the remainder being non-pyramidal. In contrast, in the more rostral prefrontal areas (which are lightly-stained), labeled cells are diffusely distributed across layers II-VI, and almost all are non-pyramidal.

The laminar distribution of immunopositive cells in heavily-labeled areas matches that of the association neurons known to project between parietal and frontal cortex, and each heavily-labeled frontal area is known to be connected (but not exclusively connected) with a heavily-labeled area in PPC. This suggests that the differential ditribution of Cat-301 IR in these regions may be related to their interconnectivity.

328.10

NORADRENERGIC AXONS DO NOT EXHIBIT TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN NEOCORTEX OF MONKEYS (*MACACA FASCICULARIS AND SAIMIRI SCIUREUS*). H. J. Noack* and D. A. Lewis (SPON: J. Puig-Antich). Depts. of Psychiatry and Behav. Neurosci., Univ. of Pittsburgh, Pittsburgh, PA 15213.

In previous studies (*J. Neurosci.* 7:279, 1987) we analyzed the regional and laminar distributions of fibers immunoreactive for tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) in neocortex from normal and locus coeruleus-lesioned monkeys. These comparisons suggested that a rabbit polyclonal anti-TH antiserum and a rabbit polyclonal anti-DBH antiserum label distinct populations of axons in monkey neocortex, which presumably are dopaminergic (DA) and noradrenergic (NA), respectively. These findings have now been confirmed in dual-labeling studies using anti-TH and anti-DBH antisera raised in different species. In single- and dual-labeling studies, mouse monoclonal, sheep polyclonal and rabbit polyclonal anti-TH antisera produced similar patterns of immunoreactivity in several regions of monkey neocortex. In contrast, fibers in monkey visual, motor, somatosensory and prefrontal cortical regions labeled with rabbit anti-DBH were not visualized with a mouse anti-TH in double-labeling studies. These antisera did, however, show double-labeling of the NA neurons of the locus coeruleus. Thus, these findings indicate that anti-TH labels DA, but apparently not NA axons in monkey neocortex. Possible biological bases for this apparent selectivity will be discussed.

328.11

THE NORADRENERGIC INNERVATION OF MONKEY PREFRONTAL CORTEX. D.A. Lewis, J.H. Morrison and H.J. Noack*. Depts. of Psychiatry and Behav. Neurosci., Univ. of Pittsburgh, Pittsburgh, PA 15213 and Scripps Clinic, La Jolla, CA.

The distribution of dopamine-β-hydroxylase (DBH)-immunoreactive (IR) fibers was characterized in prefrontal cortical regions of cynomolgus (*Macaca fascicularis*) and squirrel (*Saimiri sciureus*) monkeys. The density of DBH-IR fibers was heterogeneous, both across and within cortical cytoarchitectonic regions. For example, in cynomolgus monkey, fiber density was greatest in area 8b; within this region, the medial surface had a greater density of labeled processes than the dorsal surface. Areas 9 and 24 also had a high density of DBH-IR fibers, areas 11, 12, 13 and 25 were of intermediate density, and areas 10 and 46 had the lowest density of labeled fibers. Despite these regional variations in density, the laminar distribution of labeled fibers was very similar across areas of cynomolgus prefrontal cortex. Fiber density was relatively low in layers I-IV, increased substantially in layer V, and was intermediate in layer VI. With few exceptions, the regional and laminar distributions of DBH-IR fibers in squirrel monkey prefrontal cortex were similar to those in cynomolgus monkey. Since other data suggest that anti-DBH selectively labels noradrenergic axons in monkey neocortex, the distinctive innervation patterns exhibited by DBH-IR fibers reveals the regions and layers that may be the principle sites of action of norepinephrine in exerting its effects on prefrontal cortical function.

328.12

MONKEY FRONTAL AND CINGULATE CORTEX EXHIBIT REGIONALLY HETEROGENEOUS DISTRIBUTION OF NONPHOSPHORYLATED NEUROFILAMENT PROTEIN IMMUNOREACTIVE NEURONS. M.J. Campbell, K. Cox*, P.G. Pay*, T.A. Kimber*, and J.H. Morrison. Research Institute of Scripps Clinic, La Jolla, CA

Studies in our laboratory have revealed that a distinct subpopulation of primate cortical pyramidal neurons that furnish long projections is immunoreactive to SMI-32 (Sternberger-Meyer), a monoclonal antibody that recognizes nonphosphorylated neurofilament proteins. These studies have also revealed that the size, density and laminar distribution of SMI-32-ir neurons differ substantially across cortical areas in the temporal and occipital lobe in the primate. A variation in the quantity of neurons that are likely to furnish long corticocortical projections accounts for the major differences in the distribution of SMI-32-ir neurons across cortical areas. The present study reports results of an immunohistochemical analysis of the regionally heterogeneous distribution of SMI-32-ir neurons in frontal and cingulate cortex. Ventral medial frontal and anterior cingulate areas exhibited a striking paucity of SMI-32-ir neurons in the supragranular layers and were distinguished from each other by differences in the density of SMI-32-ir neurons in their infragranular layers. In contrast, other frontal areas and posterior cingulate could be readily distinguished from each other by substantial differences in the density of SMI-32-ir neurons in their supragranular layers. These observations suggest that there are significant differences in the laminar organization of specific classes of efferent neurons in frontal and cingulate cortical areas.

328.13

THE ChAT AND GABA IMMUNOPOSITIVE NEURONS IN THE RAT FRONTAL CORTEX: DISTRIBUTIONS IN ADULT AND AGING RATS, AND SPECIES DIFFERENCES. Y. Nishimura, M. Natori and M. Mato (Spon. Y. Inoue) Dept. Anatomy, Jichi Med. Sch., Tochigi, Japan 329-04.

We have investigated the distribution of Choline acetyltransferase immunoreactivity in neuron cell bodies (ChAT-IR) and Gamma aminobutyric acid immunoreactivity (GABA-IR) in the rat frontal cortex. This paper in the mainly reports the three dimensional distribution of ChAT-IR and GABA-IR in adult and aging rats. However in addition, the immunoreactivities against ChAT or GABA were investigated in the frontal cortex of rodents, lagomorphs, insectivores and primates.

In total seven adult rats, four aging rats (older than 2.5 years) and more than two each of golden hamsters, brown rats, house mice, guinea pigs, chipmunks, rabbits, musk shrews and squirrel monkeys were examined.

In adult rats, ChAT-IR was found to be accumulated at the lateral part of the frontal pole. The ChAT-IR then diminished caudally. GABA-IR was not found in an area matching to the accumulated area of ChAT-IR in the frontal pole. However excluding this area, GABA-IR was distributed rather evenly. These results suggest that the distribution of ChAT-IR and GABA-IR may be interrelated.

In aging rats, loss of ChAT-IR was found in the caudal part in the frontal cortex. GABA-IR sites also had a tendency to diminish in number. In Nissl preparations neuron loss was not so obvious compared with the loss of ChAT-IR with aging. This therefore suggests that the loss of immunoreactivity is not caused by neuron loss but by a synthesis disability of enzymes or neurotransmitters in the neurons of aging rats.

Of the other animals examined, ChAT-IR appeared only in the cortices of brown rats, house mice, golden hamsters and guinea pigs. In contrast, the GABA-IR was found in all of animals surveyed here. As far as we know, ChAT-IR is not found in mammals other than studied rodents. And therefore it is possible that the ChAT-IR may have been acquired specifically in rodents during their evolution.

328.14

INSTRUMENTAL EYE BLINK CONDITIONED REFLEX IS INDEPENDENT OF ASSOCIATION CORTEX IN CATS. *S.Song*, J.Harrison, J.Buchwald, Brain Research Institute, Mental Retardation Research Center, Dept. of Physiol., UCLA, Los Angeles, CA 90024.*

Multimodal association cortex (anterior lateral, peri-cruciate and medial suprasylvian areas) is not essential to classical conditioning of eye blink with auditory discrimination in cats (Norman et al., Science 196: 551, 1977). In order to examine the relation between association cortex and instrumental eye blink conditioning with auditory discrimination in cats, we used a 4KHz CS+ (conditioned stimulus) tone with duration of 400-520ms, 30DB and 60DB clicks or 1KHz and 2KHz tone pulses as CS-, and a shock US(unconditioned stimulus) delivered by electrodes at the outer margin of the orbicularis oculi muscle. We scored the CR (conditioned response) as a blink occurring 100 ms after CS onset but prior to the US. Daily training sessions consisted of 4 blocks of 25 CS+ per block over 12 sessions. Nine out of 12 cats were conditioned (≥80% CR in one block for at least 2 successive blocks). Surgical ablation of some or all areas of association cortex was performed bilaterally on all 12 trained cats. The lesions were histologically verified. Twelve sessions were run postoperatively. All 9 preoperatively conditioned cats remained conditioned. Of the 3 unconditioned cats, 2 developed CRs at the criterion level, and 1 remained unconditioned. The present study demonstrates that multimodal association cortex is not essential for learning and maintaining this instrumental conditioning of the eye blink in cats. Supported by USPHS grants HD05958 and NS25400.

329.1

SUBDIVISIONS OF VISUOMOTOR AND VISUAL CORTEX IN THE FRONTAL LOBE OF PRIMATES: THE FRONTAL EYE FIELD AND THE TARGET OF THE MIDDLE TEMPORAL AREA. J. H. Kaas and L. A. Krubitzer. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

Eye movements evoked by microstimulation, interconnections with the middle temporal visual area (MT), and myeloarchitecture distinguish at least two and possibly three subdivisions of visual cortex in the region of the frontal eye fields of prosimian galagos and New World owl monkeys, squirrel monkeys, and marmosets. In all of these primates, injections of WGA-HRP were placed in MT of visual cortex, the region of the frontal eye field (FEF) was explored with stimulating microelectrodes and marker lesions were placed at functional boundaries. The cortex was separated from the brain, artificially flattened, sectioned parallel to the surface, and reacted for HRP or stained for myelin. Two ovals of dense myelination were apparent rostral to motor cortex in these primates. The more caudomedial oval, the FEF, had a caudal division where bilateral eye movements toward the contralateral visual hemifield were evoked by low levels of stimulation (10-50μ amps), and a rostral division where higher levels of stimulation (50-100μ amps) were needed to evoke eye movements. Injections in MT resulted in little or no label in FEF. In contrast, the more rostrolateral oval of dense myelination, the frontal ventral visual area (FV), was densely interconnected with MT. Preliminary microstimulation studies in galagos have found that high levels of stimulation could evoke eye movements from FV. The presence of the FEF and FV in both simian and prosimian primates suggests that these fields originated early in primate or even mammalian evolution, and are common to all primates. Supported by EY-02686.

329.2

CORTICAL–THALAMIC PROJECTIONS FROM A PHYSIOLOGICALLY IDENTIFIED MOTOR BLINK REGION IN PARA-SIV CORTEX STUDIED WITH PHA-L IMMUNOCYTOCHEMISTRY. K. Park* and R.S. Waters (SPON: R.B. Caldwell) Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Sch. of Med., Memphis, TN 38163.

In this report we describe results of terminal labeling in the thalamus after injection of the lectin phaseolus vulgaris leucoagglutinin (PHA-L) into a physiologically identified motor blink region in the fundus of the anterior ectosylvian sulcus (AES). Clemo and Stein (1983) designated this cortical region as Para-SIV. Neurons in this region have been reported to respond to cutaneous, visual, and auditory stimuli. Our findings show that this region also contains motor output neurons that can be activated with low intensity stimulating currents to produce contractions of the orbicularis oculi muscles controlling the motor blink response.

Adult cats were prepared under inhalation anesthesia. The dura over the ectosylvian gyrus was removed, and the brain surface was exposed. The gas anesthesia was turned off, and Ketamine and Nembutal anesthetics were administered throughout the remaining part of the experiment. Tungsten-in-glass stimulating electrodes were inserted into the bank of the AES, and trains of cathodal pulses (12-13 pulses/train; 0.2ms duration, 300Hz, 40μA maximum intensity) were used to examine motor responses from this region. Once the motor map was completed, a 2.5% solution of PHA-L was iontophoresed into the center of the motor map using an anodal pulsed current of 5μA for 20-40 minutes. Following a survival time of 14-21 days, the animals were anesthetized and perfused with acetate buffer and borate fixatives. Brain sections were processed according to the method of Gerfen and Sawchenko (1984).

PHA-L injections were localized to the deeper layers of para-SIV around the stimulating electrode. The heaviest labeling was found in the posterior nucleus complex (POm and POi). Lighter labeling was seen in the lateral posterior nucleus (LP) and zona incerta. The present findings corroborate our earlier work showing that POm also receives a strong cortical projection from a cortical motor output region in area 5. Together, these studies suggest that the posterior nucleus complex may play an important role in motor control of the facial musculature.

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329.3

DIRECT PROJECTIONS FROM THE TONGUE AREA OF PRIMARY SENSORIMOTOR CORTEX TO THE HYPOGLOSSAL NUCLEUS AND ADJACENT RETICULAR FORMATION IN THE MACAQUE BUT NOT IN THE CAT. A. Sokoloff and T. W. Deacon. Biological Anthropology, Harvard University, Cambridge, MA 02138.

Degeneration studies have demonstrated a direct projection from the tongue area of primary motor cortex to the hypoglossal nucleus (HGN) in the macaque but not in the cat. In order to investigate the extent of cortical projections in the macaque and cat that might terminate in the HGN, multiple injections (0.5μl each) of the sensitive anterograde tracer WGA-HRP were made into cortical regions that included the primary sensorimotor tongue areas.

Following injections in the macaque axonal label is observed bilaterally in the HGN. Axonal label in the medulla is also observed bilaterally with slight ipsilateral predominance in the spinal trigeminal complex (spV), the lateral reticular formation just medial to spV and the reticular region bordering the HGN ventrally and laterally. Following injections in the cat labeled axons are not observed in either the HGN or adjacent reticular regions. Labeled axons are observed contralaterally in the lateral reticular nucleus and spV.

The present investigation confirms previous degeneration studies which identified a direct cortico-hypoglossal projection in the macaque but not in the cat. In addition the present study demonstrates in the macaque a bilateral projection to the reticular region adjacent to the HGN. The axons of this projection may turn medially to terminate within the HGN or may directly synapse upon ventrally and laterally directed dendrites of HGN motoneurons.

329.4

AXON-COLLATERALS OF PYRAMIDAL CELLS IN LAMINAE III AND V OF AREA 4 OF MONKEY'S CORTEX, REVEALED BY INTRACELLULAR HRP. R. Porter, S. Ghosh and R.E.W. Fyffe Experimental Neurology, John Curtin School of Medical Research, Canberra, 2601, Australia

Lamina V neurons of monkey's motor cortex with axons in the pyramidal tract have been stained by intracellular injection of HRP (Hamada et.al., Neurosci.Lett., 22:233-238, 1981). Six lamina V and four lamina III pyramidal neurons in area 4 of M. fascicularis were completely reconstructed after intracellular HRP injections. The lamina III pyramidal neurons differed from those in lamina V: they lacked long basal dendrites. Although axon collateral arbors of lamina III pyramidal cells varied in the extent of their distribution, 3 to 12 collaterals arose from each axon. The largest lamina III intracortical collateral arbor sent branches to all laminae and extended for at least 3mm mediolaterally. This contrasted with the axon collateral arbors of lamina V pyramidal neurons, which consistently exhibited three to five collaterals. Although the longer of these could extend for more than 1mm, the branches of lamina V axon collaterals were confined to laminae V and VI, whether or not the lamina V neurons were demonstrated to be PTN.

329.5

IPSILATERAL CORTICAL INPUT TO PRECENTRAL MOTOR AREAS OF THE NEWBORN AND MATURE MACAQUE MONKEY: A STUDY USING FIVE RETROGRADELY TRANSPORTED FLUORESCENT TRACERS. C. Darian-Smith*, S. Cheema and I. Darian-Smith.(SPON: A.W. Goodwin) Dept. of Anat., Brain Res. Lab., Univ. of Melbourne, Victoria 3052, Australia.

Cortical afferent input to the precentral cortex provides information required for the preparation, initiation and execution of movements. We are presently investigating ipsilateral corticocortical projections to several regions of the precentral cortex in order to determine; (1) the origins and distribution of afferent neurons in the newborn and adult monkey, (2) the juxtaposition of these populations, and (3) the presence of bifurcating axons.

Retrogradely transported fluorescent tracers (Fast Blue, Rhodamine latex microspheres, Diamidino Yellow, Evans Blue, Fluorogold; 3 to 5 in each animal) were injected into medial and lateral aspects of the premotor cortex (area 6), medial and lateral aspects of the primary motor cortex and the supplementary motor area (SMA).

Data pertaining to mature animals is incomplete. Preliminary results come from two monkeys delivered by caesarian section (E147 and E142), and one four day old infant.

For injections made into the SMA, labeled soma were observed immediately surrounding the injection site, in medial and lateral rostrocaudal strips involving areas 4 and 6 of the precentral cortex, in primary somatosensory cortex (S1), area 5, 7a and also in the limbic cortex, areas 24 and 23.

An injection into medial premotor cortex resulted in labeled soma in medial and lateral area 6, in a rostrocaudal strip across medial motor cortex, in rostral SMA, areas 24, 23 and lateral 5. Lateral premotor cortex injections produced additional labeling in area 7b.

Injections into primary motor cortex, resulted in labeled soma distributed in a rostrocaudal strip across both pre- and postcentral cortex, involving areas 4, S1 and 5. Labeling was also found in area 7b and SMA.

Corticocortical projections originated primarily in laminae III and V in primary sensory motor cortex and in laminae II to V in premotor cortex. Soma populations were observed as overlapping or segregated according to their cortical location. Double-labeled soma have been observed in limbic cortex which send bifurcating axons to both medial premotor area 6 and SMA (injection separation 6mm).

Results to date demonstrate a topographically organized cortical input from the primary somatosensory cortex to the motor cortex. They indicate that all major corticocortical projections to the precentral cortex, as described in recent literature for the adult, are present at least 2 weeks prior to birth. They also illustrate the presence of diverging neuronal projections from the limbic cortex to SMA and to the medial premotor area.

329.6

SOMATOTOPICAL ORGANIZATION OF CINGULATE PROJECTIONS TO THE PRIMARY AND SUPPLEMENTARY MOTOR CORTICES IN THE OLD-WORLD MONKEY. R.J. Morecraft* and G.W. Van Hoesen (SPON: T. Ritchie). Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242.

Experimental and clinical studies have suggested the presence of a motor-related field within the depths and the lower bank of the anterior cingulate sulcus. Indeed, corticospinal projections arise from this cortex. Despite this, little is known about the connections of this motor field, and in particular, its relationship to other cortical motor representations. Thus, a study was undertaken to examine the ipsilateral somatotopical distribution of cingulate afferents to MI and MII. Rhesus and cynomolgus monkeys were injected with retrograde tracers fast blue, diamidino yellow and HRP into face, forelimb, and hindlimb representations of MI and MII. Somatotopically organized afferents to both MI and MII were identified in the cortex of the lower bank and fundus of the cingulate sulcus. Labeling was spatially separate from area 4 (MI) and area 6 (MII) on the basis of many criteria. Projections to the various somatotopic representations within MI and MII arise from common cingulate regions, with those projecting to face located rostrally, hindlimb caudally and forelimb between. Afferents to MI originated primarily from layer V while those to MII originated from both layers III and V. Labeling was heaviest in MII cases. The results support the fact that a spatially separate and somatotopically organized motor field resides within the cingulate cortex that has direct projections to both MI and MII. Its location and relation to the motor cortices on one hand, and limbic cortices on the other, suggest an interplay between basic drives such as motivation and cortical motor mechanisms. (Supported by NS 14944.)

329.7

ORIGIN AND DENSITY OF CORTICOSPINAL (CST) PROJECTIONS FROM THE PREMOTOR AREAS OF MACAQUES. R.P. DUM and P.L. STRICK. VA Med. Ctr. and Depts. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse NY, 13210.

We examined the origin of CST projections from the premotor areas in the frontal lobe of macaques (macaca nemestrina) using retrograde transport techniques. All of the premotor areas in the frontal lobe project directly to both the primary motor cortex and the spinal cord. In particular, substantial CST projections originate from 3 separate regions on the medial wall of the hemisphere (the supplementary motor area in area 6 on the superior frontal gyrus; the caudal cingulate motor area in area 23c in the cingulate sulcus; and the rostral cingulate motor area in area 24c on the ventral bank of the cingulate sulcus) and from 2 regions within lateral area 6 (the arcuate premotor area in the caudal bank of the arcuate sulcus and the premotor area which surrounds the superior precentral sulcus).

To examine the density distribution of CST neurons, we divided layer V into bins 200 um on a side and determined the number of CST neurons in each bin. Regions of 'high' density were defined as containing greater than the 90th percentile of CST neurons per bin. There were large local variations in the density of CST neurons in each cortical area of the frontal lobe. Regions of high density were not confined to the primary motor cortex, but included substantial portions of each premotor area. These results emphasize the contribution of the premotor areas to the corticospinal system. Support: VA Med.Res.Serv. and Rehab. R&D; USPHS 24328, 2957.

329.8

EXCITABILITY OF CORTICOSPINAL NEURONS DURING TONIC MUSCLE CONTRACTIONS IN MAN. B. Brouwer*, P. Ashby, G. Midroni* (SPON: J. Murphy). Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, Ont. M5T 2S8

A magnetic stimulus applied to the human scalp over the motor cortex causes a short latency contraction of contralateral limb muscles. This is presumed to result from the indirect excitation of corticospinal neurons with monosynaptic connections to motoneurons. The excitability of these cortical neurons can be estimated from the magnitude of the postsynaptic potentials produced in spinal motoneurons by magnetic stimulation. In man the characteristics of these postsynaptic potentials can be derived from changes in the firing probability of single motor units. When a subject increases the level of a sustained voluntary contraction the excitability of the corticospinal motoneurons estimated in this way become less. We conclude that the additional synaptic input to motoneurons required for a stronger sustained muscle contraction comes from fiber systems other than the population of fast corticospinal neurons activated by magnetic stimulation.

329.9

MOTOR FIELDS OF MOTOR AND PREMOTOR CORTEX NEURONS IN THE RHESUS MONKEY DURING INDEPENDENT FINGER MOVEMENTS. M.H. Schieber. Wash. U. Sch. Med., St. Louis, MO 63110.

How does the motor cortex produce independent finger movements? Microstimulation can evoke movements of a single digit (Asanuma & Rosen, 1972), but spike-triggered averaging has shown that single cortical neurons often influence more than one spinal motoneuron pool (Cheney & Fetz, 1985). To study further the processes that produce independent movements, motor and premotor cortex neurons are being examined in terms of their **motor fields**, defined here as the subset of active movements with which a given neuron discharges.

Monkeys are trained to perform relatively independent flexion and extension movements of each digit of the right hand. The monkeys insert their hand into a pistol-grip manipulandum equipped with 10 microswitches, one positioned just ventral and dorsal to each digit. Flexion or extension of a digit 2-5 mm closes the appropriate switch. Cued by an LED display, the monkey closes only one switch at a time for a liquid reward. Preliminary data are available from one monkey.

The motor fields of motor cortex neurons can be classified as: **single digit**, if the neuron discharges with movement of only one digit in one direction; **contiguous**, if discharge occurs with movement of adjacent digits in the same direction; or **broad**, if discharge occurs with movements of non-adjacent digits or with movements in opposite directions. Digits 1 (thumb), 2 and 5 have greater representations than digits 3 and 4 among neurons with single digit motor fields. But among contiguous field neurons, digit 3 is most often the field's best digit. No contiguous field neurons have been found with best digit 1 or 5. The premotor cortex, compared to the motor cortex, has relatively few neurons related to these finger movements, though all three motor field types have been observed. Support: K08-NS01150-03 to M.H. Schieber; R01-NS12777 to W.T. Thach.

329.10

THE EFFECTS OF BILATERAL COOLING OF THE SUPPLEMENTARY MOTOR AREA ON MOVEMENT-RELATED NEURONAL RESPONSES IN AREA 4 OF CONSCIOUS MONKEYS. E.M. Schmidt and J.S. McIntosh, NIH, NINCDS, Lab. of Neural Control, Bethesda, MD 20892.

The supplementary motor area (SMA) is thought to play a role in the performance of movement. One hypothesis is that the SMA may influence the responsiveness of area 4 neurons to kinesthetic stimuli (Wiesendanger, et al., Control of Posture and Movement, 331-346, '73). Previously, we (Schmidt, et al., Soc. Neurosci. Abstr. 13: 1096, '87) reported that bilateral SMA cooling did not modify the kinesthetic responsiveness of area 4 neurons. Since then, in another animal, we have found that firing rates of some movement-related area 4 neurons are modified with SMA cooling.

A Rhesus monkey was trained to flex and extend the right wrist in response to movement of a visual target on a video monitor. The monkey's hand was held in a molded form coupled to a torque motor which produced a simulated spring load. For juice rewards, the monkey was required to match a cursor coupled to wrist-movement to a target for a period of at least one second. Halfway through the random duration hold period, a 50 ms torque pulse was applied to perturb the wrist in either the flexion or extension direction. After training, the following items were implanted under pentobarbital anesthesia and aseptic conditions: 1) six bipolar EMG leads in the right forearm muscles; 2) a recording chamber over the arm area of the contralateral precentral cortex; 3) a cooling chamber placed within the sagittal fissure overlying the territory of the SMA; and 4) a head restraint device.

Thus far, the firing patterns of 22 task related area 4 neurons were analyzed during movements, hold phases and perturbations, before, during and after SMA cooling. 77% of these neurons changed their firing patterns during cooling. Premovement burst activity was reduced during SMA cooling while low activity during a hold phase was increased. No obvious modifications of the kinematic parameters of movement or torque perturbation responses were observed with cooling.

Under the test conditions employed, cooling midline structures including the SMA appears to modifiy the movement-related firing patterns of some area 4 neurons, but the consequences of this modification on motor performance are unclear.

329.11

INTERACTIONS OF BILATERALLY DRIVEN CORTICAL OUTPUTS ON MOTOR ACTIVITY IN THE RAT. D. Asdourian, O. Hnatczuk* and S. I. Lentz*. Dept. of Psych., Wayne State Univ., Detroit, MI 48202.

The effects of bilateral electrical stimulation of the frontal cortex (Cx) on motor activity in albino rats show that the electrically driven outputs from the two hemispheres interact in ways specific to the relative intensities of stimulation of the two sides and, in some cases, to the sequence of Cx stimulation. Motor activity was recorded bilaterally from the trapezius pars cervicalis which is concerned with shoulder and forelimb movement. After the thresholds for driving activity in trapezius were established for each hemisphere in each animal, the following results were obtained: (1) When both Cxs were stimulated simultaneously with subthreshold current the effects of the stimuli summed and drove activity bilaterally, (2) When one Cx was stimulated with a suprathreshold current and the other simultaneously stimulated with a subthreshold current, summation was blocked - activity was driven only by the suprathreshold stimulation, (3) When both Cxs were stimulated with subthreshold current but stimulation was presented successively rather than simultaneously, activity was driven only from the Cx stimulated first. This last effect was seen in about 1/3 of our animals.

329.12

OLFACTION DIRECTS SKILLED FORELIMB REACHING IN THE RAT. I. Q. Whishaw and J. Tomie*. Dept. of Psychology, University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4

Rats were trained to reach for food pellets that varied in size from 20 to 300 mg from one of 21 locations on a shelf located just outside a test cage, after a barrier was lifted. Control rats walked along the front of the cage sniffing to locate the food. Once they located a food pellet, they inserted their nose between the bars of the cage, sniffed the food for one to three respiratory cycles, raised their snout slightly, and then inserted a forepaw to grasp the food. Performance was unchanged on all measures after vision was occluded with eye patches. Following olfactory bulb removal, however, the rats no longer sniffed to locate the food or sniffed the food prior to initiating a reach. Rather they systematically moved along the length of the cage inserting their snout between the bars at each possible food location and without sniffing reached for the food "as if blind". Additional impairments were not produced when the bulbectomized rats were given visual occlusion. The study demonstrates that rats locate food and direct reaching using olfaction, and are discussed in terms of the sensory control of skilled limb use and their relevance to the evolution of the sensory guidance of limb use.

329.13

SAFETY STUDIES OF MAGNETIC AND ELECTRICAL TRANSCRANIAL STIMULATION IN MAN. J. H. Kim, W. L. Levy, J. Oro, D. Tucker. Departments of Neurological Surgery University of Miami School of Medicine, Miami, FL. 33136, and University of Missouri-Columbia, Columbia, Missouri 65212.

A device for non-noxious transcranial stimulation has been introduced using a magnetic coil to induce a current in the brain. We have evaluated the safety of this method in an ongoing study from which we report data on an initial 8 volunteers. A Novametrix stimulator was used and each volunteer was stimulated 4 times in each of 4 locations, right temporal, right frontal, left temporal, and left frontal (70uV pulse, single stimuli). APB and anterior tibial EMG was recorded. Prior to and after EEG, the following tests were done: serum prolactin and cortisol, Boston naming test, story recall test and other psychometric testing. Pre and post stimulation tests showed no abnormalities. Tests during stimulation showed a marginal effect for degradation of serial 3 counting ($p=0.03$) and a significant effect of inacurracies in naming days of the week ($p=0.007$) usually omiting a day just after the stimulus. This supports a lack of long term effects, but a possible short term effect which may be usable in neuropsychological testing. We are also evaluating electrical stimulation and object movement.

SYNAPTOGENESIS II

330.1

EXTRACELLULAR GLYCOPROTEINS AT ACH RECEPTOR CLUSTERS OF CULTURED RAT MYOTUBES. G.Dmytrenko*, G.Poiana* and R.Bloch. Depts. of Neurology and Physiology, Univ. of Md. Sch. of Med., Baltimore, MD 21201

Cultured neonatal rat myotubes develop clusters of ACh receptors (AChR) where they adhere to the substrate. These clusters are often linear, with domains rich in AChR ("AChR domains") alternating with AChR-poor membrane domains that are closer to the tissue culture substrate ("contact domains"). We have used sequential detergent extraction and immunofluorescence microscopy to localize extracellular matrix components at these AChR clusters. N-CAM is present at both AChR and contact domains. Laminin and collagen IV are enriched at AChR domains, but are also found at contact domains. Heparan sulfate proteoglycan and fibronectin are present almost exclusively at AChR domains. Antibodies to rat muscle cell adherons recognize components of contact domains. These results suggest that extracellular components at substrate-apposed AChR clusters are organized into distinctive domains that parallel the organization of the cluster bilayer and its associated cytoskeleton.

We thank D.Schubert, M.Lacorbiere, H.Kleinman, J.Hassell, J.Covault and C.Cornbrooks for antibodies used in this study. Supported by the MDA and NIH grants NS01255 and NS22652.

330.2

DISTRIBUTION OF ACH RECEPTORS, 43K PROTEIN AND Na^+ CHANNEL IN NEUROMUSCULAR JUNCTION AND CULTURED MYOTUBES.

B.E. Flucher* and M.P. Daniels; NHLBI, NIH, Bethesda, MD20892.

We have compared the distribution of acetylcholine receptors (AChR) the 43K protein and Na^+ channels in AChR aggregates on cultured rat myotubes and in the mature neuromuscular junction (NMJ). Ultrathin sections of rat diaphragm and embryonic brain extract treated (4-5 h) myotubes were immunogold-labeled with antibodies[1] against AChR, 43K protein and Na^+ channel for electron microscopy.

43K protein and AChR were both concentrated at the crests of the postsynaptic folds, opposite the nerve terminal. A much lower number of sites was found in deeper regions of the folds. In contrast, Na^+ channels were found at a low site density in all parts of the postsynaptic membrane, as previously suggested by immunoperoxidase staining (Heimovich et al., *J. Neurosci.* 7:2957, 1987). In rat myotubes AChR and 43K protein were colocalized in aggregates on membrane regions with a submembrane density. These aggregates were often composed of many discrete AChR rich domains. No accumulation of Na^+ channels was detected in the newly formed AChR aggregates by immunogold labeling, possibly due to lack of sensitivity of the labeling technique. Apart from the cell surface, AChRs and 43K protein were localized in concentrations similar to those in the aggregates, on a tubular cytoplasmic membrane structure within the myotubes, distinct from previously described plasma membrane invaginations. This finding suggests that the association of AChR and 43K protein is not confined to AChR aggregates on myotubes or to the postsynaptic membrane.

1 Kindly provided by S. Fuchs, S. Froehner and R. Barchi, respectively.

330.3

OPTICAL RECORDING OF SYNAPTIC INTERACTIONS BETWEEN IDENTIFIED APLYSIA NEURONS IN VITRO. T.D. Parsons, D. Kleinfeld, G.F. Raccuia*, A.L. Obaid, and B.M. Salzberg. Dept. of Physiol., U. of Penn., Phila., PA 19104 and Bell Labs., Murray Hill, NJ 07974.

The potentiometric dye RH-155 and a 12 X 12 photodiode array have provided clean optical recordings of action potentials from the somata, initial segments, and fine processes of identified Aplysia neurons in cell culture. A pair of spikes recorded from the initial segment of a LUQ cell in a single sweep is shown in A. Synaptic interactions between cultured neurons have been detected optically. The change in absorbance by the soma and neurites of a LUQ cell, co-cultured with the inhibitory presynaptic cell, L10, is seen in B. The bar indicates a 500 ms train of action potentials in the presynaptic cell. (Spike height attenuation: 44 %.) Reduction in firing frequency in the postsynaptic cell is apparent.

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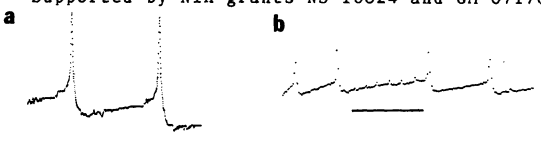


330.4

EARLY INNERVATION AND TRANSMITTER SENSITIVITY OF SYMPATHETIC NEURONS *IN VITRO*: EVALUATION OF SYNAPTIC TRANSMISSION BY ELECTROPHYSIOLOGY AND CYTOCHROME OXIDASE HISTOCHEMISTRY. R. Gardette*, G.M. Mawe, L. D'Agastaro* & L.W. Role, Dept of Anat. & Cell Biol., Ctr for Neurobiol. and Behav., Columbia Univ, P & S, 630 W 168th, NY, NY 10032

We are interested in the process of synapse formation and the regulation of transmitter sensitivity at synapses formed between neurons. To study the onset of synaptic transmission we have innervated sympathetic neurons *in vitro* with explants of preganglionic neurons (Role, PNAS, 1988) and monitored spontaneous synaptic currents and ACh-induced currents with standard whole cell voltage clamp recording.

Cholinergic synaptic potentials were detected within 24 hours. By 1 day of co-culture 42% of the cells were innervated based on recording of spontaneous synaptic currents occuring at a frequency of 0.25 ± 0.02 (n=20) Hz. Over the next 2-4 days the mean frequency of synaptic currents increased by 55-60%. The ACh sensitivity of innervated neurons was found to be 2.6 fold greater than that of non innervated cells within 24 hours of culture (289 ± 47 vs 764 ± 113 pA, n= 19). Thus, the onset of innervation and the increase in ACh sensitivity of sympathetic neurons both occur rapidly after co-culture with pre-ganglionic explants.

Activity of the cells was also monitored by measurement of the density of cytochrome oxidase (CO) reaction product (Mawe & Gershon J. Comp Neurol,1986). The early onset of synaptic transmission was reflected in increased reaction product within individual neurons. By 1 day of co-culture the reaction product density of innervated cells was increased by 2 fold compared with uninnervated cells. Increases in synaptic current frequency seen at 4 days correlated with an additional 2 fold increase in CO reaction product. The increase in density of reaction product was reversed by blockade of synaptic transmission with hexamethonium or d-tubocurare. Therefore, the density of CO staining may be a direct indicator of the degree of early innervation of individual neurons. *Supported by NS22061, Klingenstein and Sloan Foundations (L.W.R), NATO, FRM, & Philippe Foundation (RG) and NS07062 (GMM)*

330.5

THE PRESENCE OF A SYNAPTIC BASAL LAMINA ANTIGEN IN CULTURES OF NON-NEURONAL CELLS. A.Y.Chiu, S.Perez* and K.Rodriguez* City of Hope, Duarte, CA 91010

The synaptic portion of the basal lamina (BL) ensheathing muscle fibers can regulate pre- and postsynaptic differentiation during regeneration. Immunohistochemistry revealed the segregation of unique BL epitopes into synaptic and extrasynaptic domains. Interestingly, monoclonal antibodies (MAbs) C1 and C4 which selectively stained BL at the synaptic cleft also stained kidney glomerular BL. Using glomeruli as a source of antigens recognized by C1 and C4, we have partially purified a 185kd band which binds by C4 on immunoblots. Mice immunized with this purified material produced a new panel of MAbs which stain both glomerular and synaptic BL and bind epitopes different from those recognized by C1 or C4. Since synaptic BL is sandwiched between pre- and post-synaptic cells, the source of these extracellular molecules is unknown. We have begun to address this question using cultures of the rat muscle cell line, L6, as well as primary cultures of newborn rat astrocytes (gift of J. deVellis). Extracts of both cultures show a C4 immunoreactive band of 185kd. Astrocyte cultures also release the 185kd material into the medium. These preliminary results raise the possibility that a synaptic BL antigen may be produced by non-neuronal cells and then sequestered at synaptic sites where it may provide important cues during synaptogenesis. Supported by NSF grant #BNS8617043 and March of Dimes grant #5-608.

330.6

REGULATION OF SYNTHESIS OF THE 43kD POSTSYNAPTIC PROTEIN IN CHICK MYOTUBE CULTURES. D. L. Falls, D. A. Harris, R.M. Dill-Devor*, C. Carr*, J.B. Cohen*, and G.D. Fischbach. Dept of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

A *Torpedo* 43kD peripheral membrane protein is localized in a distribution coextensive with the nicotinic acetylcholine receptor (AChR) and is thought to play a role in the clustering of AChRs. An antiserum developed against SDS-denatured *Torpedo* 43kD protein labels epitopes co-localizing with the AChR at the chick neuromuscular junction. We have found that this antiserum specifically immunoprecipitates a 43kD protein from chick myotubes labeled with ^{35}S-methionine. In the experiments described here, 43kD protein synthesis was measured by labeling cultures for 4 hours and determining the amount of labeled 43kD protein precipitated per culture dish. The cultures utilized are mixed myotube/fibroblast cultures; control experiments have shown no detectable 43kD protein in pure fibroblast cultures.

The amount of 43kD protein per culture dish increased from day 1 through day 4 post-plating and then decreased between day 4 and day 7. The AChR insertion rate measured with ^{125}I-α-bungarotoxin showed a similar time course. Tetrodotoxin added at 1 μM to 6 day cultures for 24 hours consistently produced a 2 to 3 fold increase in the level of 43kD protein synthesis as well as an increase in the AChR insertion rate. However, a partially purified preparation of chicken 42kD ARIA (AChR-inducing activity), a factor that also increases the rate of AChR insertion, produced little or no increase in the amount of the 43kD protein in 4 of 5 experiments performed under similar conditions; in a fifth experiment, the level was elevated several fold. Therefore, under the conditions tested, muscle inactivity consistently increased 43kD protein, but a receptor inducing factor that may function at developing synapses did not. Current experiments are directed at determining if under other conditions ARIA does regulate the level of the 43kD protein and investigating the effects of other agents on 43kD protein synthesis.

330.7

THE RELATIONSHIP BETWEEN SYNAPTOGENESIS AND MOTONEURON SURVIVAL IN CHICK LIMB MUSCLE. L.M. Dahm & L.T. Landmesser. Dept. Physiol. & Neurobiol., Univ. Connecticut, 75 N. Eagleville Rd., Storrs, CT 06268.

Activity blockade has been shown to rescue motoneurons and to increase the number of nerve side branches in the chick iliofibularis muscle. To assess the importance of synaptogenesis itself in motoneuron survival, we examined the temporospatial distribution of pre- and postsynaptic profiles in this muscle using MAb SV2 to stain synaptic vesicles and MAb35 to stain ACh-R clusters. Although this muscle contracts in response to nerve stimulation by St 28-29, co-localization of SV2 and MAb35 staining was not observed until St 32-34. The initial SV2 staining was distributed diffusely thoughout the unbranched nerve trunks. ACh-R clusters appeared within several hours after nerve ingrowth. Although clusters were located within diffusional distance of the nerve trunks, they were not co-localized to sites of SV2 staining, suggesting an early diffuse form of synaptic transmission. Co-localization was first observed following nerve side branch formation near the onset of cell death when SV2 became localized to side branches and ACh-R clusters near nerve trunks disappeared and accumulated around side branches. The incidence of co-localization, which was restricted to side branches, increased throughout the cell death period. A similar but earlier co-localization in curarized embryos suggests that this process is not activity dependent and may be required for motoneuron survival. Supported by NIH grant 5R01 NS19640.

330.8

ELECTRICAL COUPLING BETWEEN NERVE AND MUSCLE CELLS OF XENOPUS IN CULTURE.
S.H. Young, I. Chow, and A.D. Grinnell. Jerry Lewis Res. Ctr., UCLA School of Medicine, Los Angeles, CA 90024.

The reports by Fischbach (Dev.Biol. 28: 1972) and Bonner (Dev.Brain.Res.38:1988) of electrical coupling between nerve-muscle contacts of cultured chick cells and that of Allen (J.Physiol. 372: 1986) of dye coupling between nerve-muscle contacts of cultured Xenopus cells prompted us to test Xenopus cultures for electrical coupling. The whole cell patch clamp was used in conjunction with standard microelectrode techniques producing resolution high enough to record current through single gap junction channels.

We used spontaneously occurring nerve-muscle contacts, or those which we created by manipulation of the muscle cell onto the nerve cell. Electrical coupling, measured by the flow of current from the nerve to the muscle was observed in a small fraction of nerve-muscle contacts. It remains a possibility that opened gap junctions, while not ubiquitous, may participate in inductive interactions associated with synaptogenesis. Supported by grants from the MDA, NSF, and NIH.

330.9

CELL SURFACE CONTACT INTERACTION DURING INITIAL STAGES OF SYNAPTOGENESIS. I.Chow, C.Haubach* and A.D.Grinnell. Jerry Lewis Neuromuscular Research Ctr. and Dept. of Physiology, UCLA School of Medicine, Los Angeles, CA 90024.

Direct cell-cell contact is important in synaptogenesis. There is evidence that this is enhanced by interaction between specific surface molecules, eg. neural cell adhesion molecule (NCAM), developmental regulation of which influences the timing or probability of synaptogenesis.This study was directed to assay the effect of inhibition of NCAM homophilic binding on the initial establishment of neuromuscular transmission.

When anti-NCAM (kindly supplied by Dr. U. Rutishauser) was added to Xenopus nerve-muscle co-cultures for 1-3 days, MEPPs were detected in only 32% of the nerve-muscle cell pairs, whereas 60-70% of the pairs were MEPP-positive in non-immune serum treated and control cultures. When the cells were cultured in normal medium, muscle contact-induced release of ACh from isolated cholinergic neurons (normally 70%) was inhibited by brief treatment (1 h) of anti-NCAM. Only 40% of the identified cholinergic neurons released ACh upon muscle contact. These preliminary results suggest that NCAM binding may be important in the intercellular recognition and initiation of contact-induced neurotransmitter release in this system.

(Supported by grants from MDA and NIH)

330.10

IN SITU HYBRIDIZATION OF TUBULIN α-1 mRNA AS A MARKER OF NEURONS PARTICIPATING IN REACTIVE SYNAPTOGENESIS. J.W. Geddes,[1,2] C.W. Cotman,[2] and F.D. Miller[3]. Depts. of [1]Surgery and [2]Psychobiology, Univ. of Calif., Irvine, CA 92717, and [3]Dept. Anatomy and Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada.

Reactive synaptogenesis requires the extension of neuritic elements from neuronal populations which contribute to the sprouting response. It has previously been demonstrated that the mRNA for one isotype of α-tubulin, tubulin-α1 (Tα1), is expressed at high levels during the developmental extension of neuronal processes. The level of expression decreases in the adult brain, but can be reinduced during regeneration of facial motor neurons following a nerve crush paradigm. In contrast, the level of expression of a second α-tubulin isotype, Tα26, is unchanged during development and regeneration (Miller, F.D. et al., *J.C.B.* 105: 3065-3073, 1987). To determine if neurons undergoing reactive synaptogenesis increase their expression of Tα1 mRNA, we examined the intensity of the *in situ* hybridization signal to Tα1 mRNA at various times following lesions of the entorhinal cortex, utilizing ^{35}S-radiolabelled anti-sense RNA probes transcribed from cDNAs cloned in pGEM4 vectors. In rats which had received a unilateral entorhinal lesion, an increased hybridization intensity was apparent in ipsilateral entorhinal neurons adjacent to the lesion. The increase in hybridization intensity was apparent as early as one day postlesion, greatest at 5-7 days postlesion, and was still apparent although much reduced at 14 days postlesion. These results demonstrate that similar molecular events may underlie neurite extension in development, regeneration, and synaptogenesis. The results further suggest that *in situ* hybridization of Tα1 mRNA may be a useful marker of synaptogenesis in human disorders including Alzheimer's disease, Down's syndrome and epilepsy. (Supported by the ADRDA. J.W. G. is a National Down Syndrome Society Science Scholar. F.D.M. is an AHFMR Scholar).

330.11

CHANGES IN DISTRIBUTION OF PEANUT AGGLUTININ-BINDING MOLECULES IN DEVELOPING FROG NEUROMUSCULAR JUNCTIONS. C.-P. Ko, D.B. Folsom*, and M.M. Junge*. Dept. of Biol. Sci., Univ. Southern California, Los Angeles, CA 90089.

Peanut agglutinin (PNA) specifically recognizes glycoconjugate(s) in the extracellular matrix at frog neuromuscular junctions (NMJs). To examine the role of these PNA-binding molecules (PNA-BMs) in synaptogenesis, rhodamine- or HRP-conjugated PNA was applied to muscles in tadpoles and bullfrogs (R. catesbeiana). In early stages of development, PNA-BMs are distributed over the myofiber surface. Light microscopy shows about 50% of junctions identified with alpha-bungarotoxin staining have no PNA staining distinct from the background. As development progresses, PNA background staining is reduced. Around metamorphosis, >80% of junctions show colocalization of alpha- bungarotoxin and PNA stains. PNA also stains nerve trunks in tadpole muscles, unlike in adult muscles. Electron microscopy reveals HRP reaction products at the synaptic cleft between developing nerve terminals and muscle fibers before Schwann cells are present. After Schwann cells appear, reaction products still are confined to the cleft, but later appear also in the extracellular matrix around Schwann cells, as in adult junctions.

Thus, in synaptogenesis, PNA-BMs initially are seen over the nerve and muscle surfaces but later are confined to NMJs, first in the cleft, then also around Schwann cells. The appearance of PNA-BM in the initial nerve-muscle contact is not dependent on Schwann cells.

330.12

SYNAPTIC DEVELOPMENT INHIBITED BY NMDA ANTAGONISM W.J. Brooks*, T.L. Petit, R. Lo *, and J.C. LeBoutillier*. Dept. of Psychology, Prgm. in Neuroscience, Univ. of Toronto, Ont., Canada M1C 1A4

Synaptic plasticity induced by neuronal activation is thought to provide a physiological basis for learning and memory. Experimental models of activity dependent enhancement in neurotransmission have reliably found alterations in a number of synaptic parameters. Recently, activation of the NMDA receptor has been shown to be a critical step in the production of a plastic response by the synapse.

Over the course of development, the rapid increase in synaptic number is thought to correspond to the expansion of behavioral repertoire in response to the postnatal environment. Therefore, developmental synaptogenesis is also a model of learning and memory. The possible involvement of the NMDA receptor in developmental synaptogenesis was investigated using the selective NMDA antagonist DL-2-amino-5-phosphonovaleric acid (APV). Fifteen day old rat pups were intracranially administered APV via osmotic pumps. The pups were sacrificed two weeks after implantation (P30), cortical sections dissected out and processed for electron microscopy. Photographic analysis revealed a decrease in the number of synapses within the molecular layer of cerebral cortex in rats administered APV. This finding suggests that developmental synaptogenesis results from a mechanism similar to that producing synaptic plasticity in the adult.

330.13

REGULATION OF NEUROMUSCULAR JUNCTION DEVELOPMENT BY MUSCLE ACTIVITY. E. Bornslaeger*, G. Marazzi*, and L.L. Rubin. (SPON: J. Adler). Rockefeller Univ., NY, NY 10021.

Three important changes that occur during neuromuscular junction development are an increase in acetylcholinesterase (AChE) levels, a decrease in the level of extrajunctional acetylcholine receptor (AChR), and an increase in AChR half-life ($t_{\frac{1}{2}}$). Previously, we found that muscle activity regulates AChE and AChR levels, apparently by increasing intracellular Ca^{2+} (Rubin, L.L., PNAS 82: 7121, 1985). Tetrodotoxin (TTX) inhibits contraction of cultured rat myotubes and prevents an increase in AChR levels; these effects are overcome by simultaneous treatment with Ca-ionophores. Veratridine, which opens sodium channels but blocks contraction, promotes an increase in AChE, probably by stimulating a plasma membrane Na/Ca exchanger. Here, we report that muscle activity also appears to regulate AChR degradation rate. Treatment with TTX decreases AChR $t_{\frac{1}{2}}$ relative to untreated cultures. Effects of TTX were overcome by the Ca-ionophores A23187 or ionomycin, which cause release of intracellular Ca and an influx of Ca through plasma membrane channels. Veratridine also increased $t_{\frac{1}{2}}$ and decreased AChR levels. Curiously, ryanodine, which releases Ca from the SR, altered AChR and AChE levels, but did not affect AChR $t_{\frac{1}{2}}$. This suggests that AChR and AChE synthesis can be modulated by Ca influx through plasma membrane channels as well as Ca release from the SR. AChR $t_{\frac{1}{2}}$, however, appears to be regulated only by the former mechanism.

330.14

INVOLVEMENT OF A 37 kD PROTEIN IN AChR CLUSTERING. G. Marazzi*, F. Bard*, L.L. Rubin. Rockefeller Univ. NY, NY 10021.

Our laboratory has shown that chick muscle cells transformed with Rous sarcoma virus are unable to cluster acetylcholine receptors (AChRs) even after treatment with a clustering factor (CF) derived from Torpedo electric tissue (Anthony et al., PNAS 1984, 81, 2265-2269). Transformed cells are missing a 37 kD protein that is recognized by an antibody against nonmuscle tropomyosins (Anthony et al., J. Cell Biol., in press). This protein is concentrated at rat neuromuscular junctions. To determine if this protein is involved in the formation and/or maintenance of AChR clusters, we microinjected a monoclonal antibody (D3-16) against it into cultured muscle cells. After injection, cells were treated with CF, incubated with rhodamine α-bungarotoxin to localize AChRs, then fixed and labeled with FITC-conjugated second antibody to identify injected cells. D3-16 injected cells had a decreased ability to form AChR clusters; the antibody did not disassemble the preexisting clusters. Cells injected with nonimmune mouse immunoglobulin (IgG) were still able to form new clusters. To further test the role of the 37 kD protein, we disrupted clusters with sodium azide before injection. After azide washout, noninjected and nonimmune IgG-injected cells could form new clusters, but D3-16 injected cells could not. These data suggest that the 37 kD protein may play an important role in the formation of AChR clusters, probably as part of a sub-cluster cytoskeletal network.

TROPHIC AGENTS IV

331.1

EFFECTS OF BASIC FGF AND NGF ON SEPTAL NEURONS AFTER FIMBRIA FORNIX TRANSECTION AND IN CULTURE. D. Otto*, M. Frotscher*, C. Grothe* and K. Unsicker. Depts of Anatomy and Cell Biology, Univ. of Marburg, and Anatomy, Univ. of Frankfurt, F.R.G.

Basic FGF and NGF are present in the brain and trophic functions for the maintenance and transmitter metabolism of several CNS neuron populations have been assigned to them. We report here that both NGF and bFGF are capable of reducing neuronal death in the medial septum after unilateral fimbria fornix transection (FFT) and enhance survival and choline transferase activity in cultured embryonic septal cells. Adult rats received gel foam implants containing factors or vehicle, respectively, to the FFT site. Cell counts performed on cresyl violet-stained sections after four weeks revealed dramatic neuron losses (87%) on the lesioned as compared to the unlesioned sides, which were significantly reduced by NGF-(0.3 µg: 71%; 19 µg:54%) or bFGF- (8.6 µg:68%) treatments. Both factors sustained substantial proportions of Chat-immunoreactive neurons. NGF and bFGF also significantly increased Chat activity in septal neurons cultured from E16 rat embryos. Promotion of survival was only seen in low density cultures, but comprised both cholinergic and GABA-ergic neuronal subpopulations. These data suggest a trophic role of bFGF for septal neurons in vivo and in vitro.

Supported by grant Un 34/13-1 from the German Research Foundation.

331.2

LOCALIZATION OF NGF RECEPTOR mRNA IN THE RAT FOREBRAIN USING IN SITU HYBRIDIZATION. R.B. Gibbs[1], J.T. McCabe[1], C.R. Buck*[2], M.V. Chao*[2], and D.W. Pfaff[1]. [1]Lab of Neurobiology and Behavior, Rockefeller University, New York, N.Y. 10021, [2]Department of Cell Biology and Anatomy, Cornell University, New York, N.Y., 10021.

The expression of nerve growth factor receptor (NGFr) mRNA was examined in the adult rat CNS through the use of in situ hybridization. Sense and antisense ^{32}P-labeled riboprobes were transcribed from a 320 bp EcoR1-BamH1 fragment of the rat NGF receptor gene inserted into a pT3/T7 transcription vector.

Adult, Sprague Dawley rats (350-450 g) were used. The brains and superior cervical ganglia (SCG) were removed, blocked, and frozen in liquid nitrogen. Eight micron frozen sections were cut and mounted onto diethylpyrocarbonate-treated, gelatin-coated slides. The sections were fixed with 4% paraformaldehyde/PBS, rinsed, dehydrated and stored in a desiccator at room temperature overnight. Sections were hybridized with 10-50 ng sense or antisense probe (5 x 10^8 cpm/µg) for 40 h at 23-50°C. Sections were then treated with RNase-A (2.5-10 µg/ml), rinsed with SSC, dehydrated, dipped in Kodak NTB3 emulsion and stored at 4°C. After 10-30 days exposure, autoradiograms were developed and counterstained with cresyl violet.

The most intense cellular labeling was observed over neurons in the SCG and in areas of the septum, diagonal band, preoptic and ventromedial nuclei of the hypothalamus, the bed nucleus of the stria terminalis, the oculomotor nucleus, and the midbrain central gray. A few scattered cells were also observed in the striatum, cerebral cortex, red nucleus, and in the retrorubral field. Cellular labeling was not observed in sections hybridized with sense probe (probe complimentary to the NGFr gene). We are currently examining other areas of the CNS while continuing to optimize hybridization conditions necessary to detect mRNAs produced in very low quantities in the brain.

Supported by NIH postdoctoral grant #NS08195.

331.3

EFFECT OF NERVE GROWTH FACTOR IN THE ELECTRICAL MEMBRANE PROPERTIES OF HUMAN FETAL DORSAL ROOT GANGLIA NEURONS IN CULTURE. P. Caviedes* and S.I. Rapoport (SPON: A. Noronha). NIH, NIA. Lab. of Neurosciences. Bethesda, MD 20892.

Nerve growth factor (NGF) is a peptide that reportedly enhances survival of embryonic mice dorsal root ganglia (DRG) neurons and affects the duration of the action potential. In PC12 cells, it has been shown to induce the production of Sodium channels.

In the present study, fetal human DRG neurons obtained from abortuses of 16 to 19 weeks of gestation, were cultured in absence and presence of 40 nM NGF. After 1 week in culture, action potentials were recorded using the whole cell patch-clamp technique, in current clamp mode. Current steps of 0.1 nAmps with durations of 2 and 100 milliseconds were employed. At similar resting potential levels, cells grown in presence of NGF showed faster maximal rates of depolarization (+55.2%), of repolarization (+54.6%) and shorter duration (-20.6%) of the action potential compared to controls. The results indicate that NGF regulates the electrical activity of human fetal DRG neurons in culture.

331.4

FURTHER ANALYSIS OF NGF EFFECTS IN RATS WITH PARTIAL FIMBRIAL TRANSECTIONS. C.N. Montero*, D.C. Mash, E.O. Junard* and F. Hefti (SPON: W.L. Strauss). Dept. of Neurology, University of Miami, Miami, FL 33101.

We have previously shown that intraventricular NGF administration to adult rats with partial fimbrial transections prevents the lesion-induced disappearance of septal cholinergic neurons. NGF treatment was now found to be equally effective in 18 months old female rats. Unilateral fimbrial transections reduced the number of ChAT and NGF receptor positive neurons in the septal area by 65% in old animals. In NGF treated old rats the decrease in cell number was only 11%. We also addressed the question whether the disappearance of cholinergic cell bodies represents morphological degeneration or simply down-regulation of cholinergic marker enzymes and NGF receptors. Septo-hippocampal neurons were retrogradly labeled with fluorogold before lesioning the fimbria. Preliminary findings indicate that the lesion reduces the number of fluorogold labeled, AChE-positive neurons in the septum, suggesting cellular death after lesioning. Long-term but not short-term NGF treatment permanently rescued the cholinergic cell bodies from lesion-induced degeneration and stimulated regrowth of cholinergic fibers into the denervated hippocampus, suggesting that, after long-term treatment, the lesioned neurons had again gained access to their source of endogenous NGF.

331.5

NERVE GROWTH FACTOR REGULATES THE ELECTROPHYSIOLOGICAL PROPERTIES OF MATURE FROG SYMPATHETIC NEURONES. P. Traynor*, W.F. Dryden & P.A. Smith, (SPON: S. Malhotra). Dept. Pharmacol., Univ. Alberta, Edmonton, Canada

Axotomy of B-cells in bullfrog sympathetic ganglia (BFSG) results in an increase in action potential (AP) duration (spike width) and a reduction in the amplitude and duration of the afterhyperpolarization (AHP) which follows the AP (Kelly et al., Neurosci. letts. 67:163, 1986). These axotomy-induced changes may be due to the loss of the retrograde transport of nerve growth factor. (NGF; Levi-Montalcini, Prog. Brain. Res. 45:235, 1976). 2.5s NGF (50 ng/ml) enhances spike width and restores AHP amplitude and duration towards control levels in explant cultures of (axotomized) BFSG neurones (Traynor et al., Soc. Neurosci Abs, 13:1441, 1987). This finding supports the hypothesis that part of the electrophysiological response to axotomy results from a loss of a retrograde supply of NGF. Schwann cells produce NGF, thus axotomized neurones may have access to some NGF (Abrahamson, Dev. Brain Res., 27, 117, 1986). When anti-NGF (affinity isolated sheep IgG, 0.5 μg/ml) was included in the culture medium of BFSG explants, in order to neutralize endogenous NGF, there was an enhancement of the electrophysiological effects of axotomy. These results suggest that the electrophysiological properties of mature, BFSG neurones is under control of NGF.

Supported by MRC (Canada) and AHFMR.

331.6

A BETTER CONTROL OF INTRAVENTRICULAR NGF INFUSION IMPROVES THE PROTECTION OF AXOTOMIZED CHOLINERGIC MEDIAL SEPTUM NEURONS. H.L. Vahlsing*, T. Hagg, S. Varon, M. Manthorpe (SPON : D.R. Brady). Dept. Biology, Univ. of California, San Diego, La Jolla, CA 92093.

Intraventricular Nerve Growth Factor (NGF) infusion prevents the disappearance of axotomized adult rat medial septum cholinergic (MSC) neurons. We had previously developed a small 33 gauge (ga) cannula device for continuous infusion of NGF or other substances into the lateral ventricle at the level of the rostral medial septum (Williams, L.R. et al., Exp.Neurol. 95:743, 1987). The device consists of a metal cannula embedded in a dental acrylic stabilization platform and connected by vinyl tubing to an Alzet 2002 minisosmotic pump. When NGF was infused during the 2 weeks following fimbria-fornix transection, protection varied between 60 and 100% in different animals. After the 2 week pumping period, the flow-rate was also found to vary and often be very low compared to the pre-implantation rate, -- raising the concern that the small inner diameter of the cannula allowed it to become progressively clogged. To secure a constant infusion, the small 33 ga cannula was replaced by a larger 30 ga one, whose outer diameter was reduced to about 32 ga by acid treatment. With this new cannula the protection by NGF of the axotomized MSC neurons was improved to a consistent 95-105%. However, the prolonged infusion at higher flow rates induced a lesion in the tissue around the cannula tip. Partial paraffin coating of the pump surface resulted in a reproducible reduction of the flow rate and an elimination of the infusion-related lesion. Using the same dosage of NGF infused through the 32 ga cannula at the reduced flow rate still resulted in the same degree of MSC neuronal protection. Supported by NIH grant NS 16349 and APA grant TC 88-01

331.7

ALPHA$_1$-ADRENERGIC RECEPTORS AND PROTEIN KINASE C STIMULATE NGF RELEASE FROM THE SUBMAXILLARY GLAND. C.L. Hix and J.N. Davis. Depts. of Pharmacology and Medicine (Neurology), V.A. and Duke Univ. Med. Centers, Durham, NC 27705.

Although much research has been done to determine the tissue distribution and biological action of NGF, very little is known about what regulates NGF release. We are testing the hypothesis that receptors which activate protein kinase C (PKC) stimulate NGF release from target tissues. We began by examining the effect of stimulating adrenergic receptors on NGF release from the male mouse submaxillary gland. NGF was measured using a one-site ELISA. Dispersed submaxillary cells show a slow release of NGF over time and increased release when stimulated with epinephrine ($p<0.003$ compared to buffer). The release stimulated by 10 μM epinephrine is completely blocked by 0.15 μM prazosin. In contrast, 1 μM yohimbine or 0.2 μM propranolol only partially blocked the release. Thus, stimulation of alpha$_1$-adrenergic (α_1) receptors causes NGF release from the male mouse submaxillary gland. The effect of stimulating PKC on NGF release was tested using phorbol 12-myristate 13-acetate (PMA). Cells incubated with 100 μM PMA for 60 min. released significantly more NGF than cells incubated with buffer. This data is consistent with the hypothesis that NGF release in this tissue is induced by stimulating α_1 receptors which activate PKC.

This work was supported by the V.A. and N.I.H. (NS06233).

331.8

NGF ACTIVATION OF PROTEIN KINASE ACTIVITY AND C-FOS TRANSCRIPTION IS INHIBITED BY K252a. Gary E. Landreth, Deanna S. Smith*, Carolyn S. King*, Eric Pearson* and Cynthia Gittinger*. Department of Neurology, Medical University of South Carolina, Charleston, SC 29425.

NGF rapidly stimulated the activity of two protein kinases, a MAP2/pp250 kinase and a kinase which phosphorylated the peptide substrate Kemptide. Preincubation of PC12 cells with K252a, a protein kinase inhibitor, specifically blocked the ability of NGF to activate both kinases while having little or no effect on the EGF-stimulability of these activities. In vivo half-maximal inhibition was observed at 20 nM K252a, however, 2-4 fold higher levels of K252a were required to half-maximally inhibit the kinases when the drug was added to the assay in vitro. The data suggest that action of the drug in vivo was not due to a direct action on these kinases. The action of K252 was similar to that observed with methyltransferase inhibitors e.g. 5'methylthioadenosine (MTA). MTA was an effective inhibitor of protein kinases in vitro (K_i 100 μM). K252a treatment of PC12 cells blocked the NGF, but not EGF or TPA-stimulated transcription of c-fos. The data demonstrate that K252a inhibits a variety of rapid effects of NGF on PC12 cells.

331.9

ANALYSIS OF DELETION MUTATIONS IN THE NERVE GROWTH FACTOR RECEPTOR. A.A. Welcher, M.J. Radeke*, C.M. Biler*, and E.M. Shooter. Dept. Neurobiology, Stanford U. Sch. Med., Stanford, CA 94305

Nerve growth factor (NGF) is a polypeptide that exerts its action by interacting with specific receptors on the surface of its target cells. Two populations of receptors exist on the surface of sensory and sympathetic neurons, as well as PC12 cells. The two receptor types exhibit different dissociation kinetics and steady state binding of NGF. The major population of receptors releases NGF rapidly ("fast" receptor) while the second population releases NGF more slowly ("slow" receptor). A cDNA clone for the rat "fast" NGF receptor was recently isolated. The protein encoded by the cDNA clone contains a signal peptide, four cysteine-rich elements, two putative N-glycosylation sites, and a hydrophobic rich region which is probably a membrane spanning region. In order to identify the amino acids needed by the receptor for NGF binding, we made mutations in the cDNA clone, transfected the mutants into mouse L cells, and determined the effect on NGF binding by the mutated receptors. The first mutant is a deletion mutant containing the first 706 nucleotides of the receptor clone, but lacking the portion encoding the hydrophobic rich region as well as the carboxyl end of the protein and the 3' untranslated region. This truncated receptor sequence was subcloned into an eukaryotic expression vector and transfected into mouse L cells. A truncated form of the receptor was secreted from the cells, and was recognized by an NGF receptor specific antibody, MC192, but was unable to bind to an NGF-sepharose column under conditons where the full length receptor would bind. These results suggest that the hydrophobic rich region is a membrane spanning region and is needed to anchor the receptor in the membrane. The membrane spanning region and the 3' end of the receptor might be directly involved in NGF binding or may influence the structure of the NGF binding domain. In order to further delineate receptor sequences neccessary for NGF binding, we have constructed a set of nested deletions that span several hundred nucleotides at the 5' end of the cDNA clone, commencing after the signal peptide, by exonuclease III digestion . The deleted receptor clones were subcloned into an expression vector for tranfection into mouse L cells. Characterization of these transfected cells in terms of NGF binding will be discussed.

331.10

PRODUCTION OF A POLYCLONAL ANTISERUM RECOGNIZING THE NGF RECEPTOR. D.L. Shelton and E.M. Shooter. Dept. Neurobiology, Stanford U. Sch. Med., Stanford, CA. 94305.

All known effects of nerve growth factor are mediated by interaction with a cell surface receptor (NGFR). Although there are monoclonal antibodies that recognize the extracellular domain of the rat (MC192) or primate (a series of monoclonal antibodies) NGFR, additional antibodies would be useful. First, the currently available antibodies recognize NGFR only from the rat or primate, limiting the use of other systems. Secondly, new antibodies to discreet epitopes would provide a stronger correlation between the presence of NGFR-like immunoreactivity and the presence of the NGFR. Finally, the monoclonal antibodies react poorly, if at all, with denatured NGFR, precluding the use of immunoblots.

In order to generate such antibodies, sequence information from the recently cloned cDNA's encoding the human and rat NGFR was used to define a peptide from a conserved intracellular domain of the NGFR. This peptide was synthesized and used to produce a serum in rabbits . The antibodies were affinity purified against the peptide and subjected to the following tests to determine if they recognized the NGFR. PC12 cells were iodinated, solubilized in NP-40, immunoprecipitated with MC192 or the anti-peptide serum and run on SDS gels. Each gave rise to a single labelled band of about 84 kd. In order to determine if the anti-peptide serum recognized denatured NGFR, various cell types were solubilized, run on SDS gels and transferred to nitrocellulose. When the transfers were probed with the anti-peptide serum, nothing was detected in proteins from L cells, whereas a single strong band of about 84 kd was detected in proteins from L cells transfected with DNA encoding the NGFR and in proteins from PC12 cells. Using immunocytochemistry, the anti-peptide sera reacted strongly with sensory and sympathetic ganglia and with cells of the basal forebrain nuclei. Taken together, these data indicate that the anti-peptide serum recognizes the NGFR in its native, denatured and fixed state.

331.11

SOLUTION HYBRIDIZATION ASSAY TO MEASURE mRNA LEVELS FOR NERVE GROWTH FACTOR RECEPTOR. J. Hartikka, F. Hefti and W. Strauss. Depts. of Neurology and Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

We have reported that treatment of septal cultures with nerve growth factor (NGF) increases the staining intensity of cholinergic neurons visualized with antibodies against the nerve growth factor receptor (NGFR). These results suggest that NGF stimulates the synthesis of its own receptor. In order to study the regulation of NGFR expression, we have developed a method to quantify the amount of mRNA coding for this receptor using a solution hybridization assay.

A cDNA clone for the rat NGFR (Radeke et al., Nature 325: 593, 1987) was subcloned in pGEM-blue. A single-stranded cRNA probe (specific activity 8 X 10^5 cpm/ng) was transcribed using SP6 RNA polymerase. This probe was hybridized (RoT = 0.008) to a sense strand cRNA transcribed from the same clone using T7 RNA polymerase. After digestion with ribonucleases A and T1, the nuclease-resistant RNA was precipitated and the amount of specific mRNA was quantified. Based on this assay, the concentration of mRNA coding for NGFR is less than 0.1 ng/mg of total RNA in the basal forebrain of adult rats.

331.12

PURIFICATION OF A TRUNCATED HUMAN NERVE GROWTH FACTOR (NGF) RECEPTOR. A.A. Zupan*, P.A. Osborne*, and E.M. Johnson, Jr. Department of Pharmacology, Washington University, St. Louis, MO 63110.

Our laboratory recently described the presence of a truncated form of the nerve growth factor receptor (NGFRt) in the urine, plasma, and amniotic fluids of rats. We report here the presence of forms of NGFRt in human urine and amniotic fluid by utilizing the human-specific anti-NGFR monoclonal antibody 20.4. ^{125}I-NGF specifically bound to NGFRt was chemically crosslinked using a water-soluble carbodiimide. After immunoprecipitation, labelled receptor species were visualized by autoradiography following SDS-PAGE. Labelled species corresponded to proteins of approximate molecular weights 60, 50, and 37 KDa.

Employing human adult male urine as starting material, NGFRt was purified to near homogeneity by using a combination of ion exchange and immunoaffinity chromatographies. Typical yields were about one μg/L urine. The purified protein was NGFRt as shown by an NH_2-terminal sequence identical to that predicted by the known sequence of human NGFR. In addition, amino-acid composition analysis of the protein was consistent with it being the extracellular domain of the NGFR assuming a cut site at Arg-219. The purified protein was used as an immunogen to generate polyclonal antisera. These sera blocked ^{125}I-NGF binding to A875 human melanoma cells and to PC12 rat pheochromocytoma cells. Purified NGFRt may be useful in structural and immunological studies of the native human NGFR.

331.13

IN VIVO EFFECTS OF GM1 AND NGF, ADMINISTERED IN COMBINATION, FOLLOWING CNS RETROGRADE DEGENERATION. L. Garofalo*, D. Maysinger and A.C. Cuello. Dept. Pharmacology, McGill University, Montreal, Canada, H3G-1Y6.

Following a unilateral decortication, retrograde degenerative changes occur in the nucleus basalis magnocellularis (NBM) of mature rats which can be prevented by administering the monosialoganglioside GM1. Nerve growth factor (NGF) administered i.c.v. via minipumps (12 ug/day, 7 days) is equally effective. We have presently been examining the possibility that GM1 may exert its effects by enhancing the action of endogenously occuring neuronotrophic factor(s). NGF, at the above dose, administered i.c.v. in combination with a dose of GM1 (0.5 mg/kg/day, 7days) which is ineffective in this lesion , increases ChAT activity in the NBM (120%) and in particular in the remaining cortex (155%) above that obtained by NGF alone. Furthermore, NGF, with a dose of GM1 (5mg/kg/day, 7 days) which is effective in our model, increases ChAT activity in the NBM and in particular in the remaining cortex (247%) well over that obtained by these two agents alone. The effects of these agents in other brain areas and the morphological appearance of NBM cholinergic neurons following this lesion and treatments will also be presented.These preliminary studies support previous work in the PNS and suggest that GM1 may modulate the action of neuronotrophic factors in the CNS in vivo. Supported by MRC, FCAR and FRSQ.

331.14

RETROGRADE TRANSPORT OF NERVE GROWTH FACTOR BY MOTOR NEURONS OF DEVELOPING RATS: ASSESSMENT OF POTENTIAL NEUROTROPHIC EFFECTS. W. Snider, Q. Yan, J. Pinzone* and E.M. Johnson, Jr. Depts. of Neurology and Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Several regions of the central and peripheral nervous system transiently express receptors for nerve growth factor (NGF) as revealed by a specific monoclonal antibody to the rat NGF receptor (Yan and Johnson, J Neurosci, in press). In the spinal cord, staining clearly associated with ventral motor neurons is observed from about E15, peaking on the day of birth, and decreasing to undetectable levels by postnatal day 10. In order to assess the function of these receptors we have studied the retrograde transport of NGF by spinal motor neurons and the responses of motor neurons to pharmacologic doses of NGF in neonatal rats.

^{125}I-NGF was retrogradely transported by motor neurons from their peripheral nerve terminals in newborn animals. This transport was blocked by an excess of unlabeled NGF but not by cytochrome c. ^{125}I-cytochrome c was not transported. The monoclonal anti-rat NGF receptor antibody was also transported, but not a control antibody. Despite this ability of motor neurons to transport NGF, treatment of neonatal rats with this factor did not increase motor neuron size or synthesis of neurotransmitter enzymes and did not prevent cell death after axotomy. We conclude that NGF receptors of spinal motor neurons can bind, internalize, and retrogradely transport NGF. However, these receptors do not mediate the morphological and survival-promoting effects associated with the action of NGF on sympathetic and dorsal root ganglion cells.

331.15

ULTRASTRUCTURAL LOCALIZATION OF NGF RECEPTORS IN DEVELOPING RAT SKELETAL MUSCLE. H. B. Clark, C. Grgurich* and Q. Yan Dept. of Pathology, So. Ill. Univ. Sch. of Med./Memorial Med. Ctr., Springfield, IL 62781 & Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The expression of nerve growth factor (NGF) receptor was studied in embryonic rat skeletal muscle by electron microscopic (EM) and light microscopic (LM) immunocytochemistry using 192-IgG, a monoclonal antibody specific for rat NGF receptor. NGF receptor immunoreactivity (NGFRI) in skeletal muscle was seen as early as embryonic day 11 (E11) but was most intense in E15 animals. In E15 rats the NGFRI seen by EM was localized to the cell surfaces of two cell types. Myotubes both with and without clearly-defined myofibrillar cytoplasmic components had intense NGFRI. Cells that were less obviously differentiated, with a spindled, fibroblastic appearance, also were heavily immunostained. By EM the NGFRI in E18 rats was absent or only faintly present on the more well-differentiated muscle cells. At E18 there still were numerous spindle-shaped cells with intense NGFRI interspersed among the fibril-containing muscle cells. By the time of birth the NGFRI seen by LM was greatly diminished and by EM appeared to be confined to the spindle-cell population. The transient expression of NGF receptors by skeletal muscle during development appears to be correlated with the cellular differentiation of myotubes. NGF may play a role in cellular mechanisms related to the fusion of myoblasts into myotubes and subsequent myotubular differentiation into mature myocytic syncytia.

331.16

GLUCOCORTICOID REGULATION OF NERVE GROWTH FACTOR mRNA IN RAT AND MOUSE TISSUE. R. P. Hellendall*, K. E. Sheppard*, M. Blum (SPON:I. Bodis-Wollner) Fishberg Ctr. for Neurobiology, Mt. Sinai Sch. of Med., N.Y., NY 10029

We have examined the effect of glucocorticoids on nerve growth factor (NGF) gene expression in the adult rat and mouse brain. Glucocorticoids have been found to increase nerve growth factor protein content in the mouse submaxillary gland; the mechanism of action for this modulation is unknown. Our work has focused on the cortex and hippocampus, the two regions containing the highest NGF mRNA levels. Utilizing the sub-picogram sensitivity of a S1 nuclease protection assay we have found basal NGFmRNA levels in the Sprague-Dawley rat in agreement with previous studies (10 fg/ug total RNA in hippocampus, 5 fg/ug total RNA in cortex). Adrenalectomy of rats, Swiss-Webster and CB57BL mice caused a down regulation of NGF gene expression in the hippocampus whereas no consistant change was observed in cortex. One week treatment with dexamethasone (.25ug/ml in drinking water) produced an increse in NGF mRNA to a level above that of intact controls in the hippocampi of rats and CB57BL mice and in the cortex of CB57BL mice. Submaxillary tissue is currently being studied to understand the peripheral effects of the adrenalectomy/replacement paradigm. We are also examining the effects of glucocorticoids on septal NGF receptor gene regulation: preliminary data indicate adrenalectomy increases NGF-R mRNA in Sprague-Dawley rats.

331.17

THE CONSTRUCTION OF HUMAN NGF EXPRESSION VECTORS: THEIR POTENTIAL ROLE IN THE TREATMENT OF ALZHEIMER'S DISEASE. G. Bruce* and G. Heinrich, Lab. Molec. Endocrinology, Mass. Gen. Hospital, Boston, MA 02114

Nerve growth factor (NGF) has recently been implicated as a trophic agent in the survival and maintenance of the basal forebrain cholinergic neurons. Evidence from animal studies suggests that a continuous intraventricular infusion of NGF or the intracerebral grafting of NGF producing cells into the Alzheimer's brain may stop the degeneration of these neurons and improve memory function. For this purpose human NGF (hNGF) expression vectors were constructed to allow for the production of recombinant hNGF for infusion and also for the potential to transform cells for grafting. Three expression vectors were constructed containing the entire coding region for mature (13,000 MW) hNGF derived from the 3' exon (exon 4) of the hNGF gene. Vector 1, a pBR322 derived vector containing the metallothionein promoter, consists of a 780 bp Apa1-Bcl1 hNGF fragment joined to a linking oligo supplying the lacking 30 bases of the 5' end. Vector 2, a pXM derived vector containing the AV MLP promoter, consists of a 1050 bp Ava1-Apa1 hNGF fragment. Vector 3, a pGEM-3 vector containing the metallothionein promoter, consists of a 1200 bp Hind III-Bcl 1 hNGF fragment joined to a 255 bp fragment of mouse NGF cDNA. All the inserted hNGF fragments contain the two potential initiation codons at -123 and -119 with the mouse NGF cDNA fragment of vector 3 also supplying the 5' sequences up to the potential initiation codon at -187. All vectors were selected and shown to contain the respective inserts by restriction mapping and oligonucleotide hybridization. These vectors are being transiently expressed in COS, CHO and PC12 cells. Expression will be measured by analysis of the transfected cell medium for immunoreactive and bioactive NGF. It is hoped that the successful expression of hNGF may represent an important first step in the potential treatment of Alzheimer's disease.

331.18

RAT β-NGF SEQUENCE AND SITES OF SYNTHESIS IN ADULT CNS. P.L. Friedman, D. Larhammar, V.R. Holets, M. Gonzalez-Carvajal*, Z.Y. Yu*, H. Persson* and S.R. Whittemore. Dept. Neurological Surgery, Univ. Miami, Miami, FL 33176 and Dept. Medical Genetics, Univ. Uppsala, S-751 23 Uppsala, Sweden.

The nucleotide sequence of a rat β-nerve growth factor (NGF) genomic sequence encoding the entire 3' exon of preproNGF was determined. Rat NGF shows very high homology with other known NGFs in both 3' untranslated regions and the prepro-peptide. The presumptive signal sequence, cysteine residues important for tertiary structure, glycosylation sites and dibasic amino acids required for proteolytic cleavage to mature NGF are highly conserved. Comparison of hydrophobicity plots and amino acid sequences revealed an evolutionarily divergent domain on the external surface of NGF which may account for the poor immunologic cross-reactivities of the various NGFs.

In situ hybridization to brain sections with a rat-specific oligonucleotide indicated high levels of NGF mRNA synthesis in both hippocampal granule and pyramidal cell layers. These results are consistent with one role for NGF in the CNS as a neuronally-released, retrogradely transported neurotrophic factor for basal forebrain cholinergic neurons. Labelled cells were also observed in olfactory cortex and other CNS regions are presently being examined.

GENETIC MODELS II

332.1

SEVERITY OF CORPUS CALLOSUM DEFICITS IN TWO SUBSTRAINS OF BALB/cWah MICE IN RELATION TO UTERINE LOCATION OF FETUSES B. Bulman-Fleming and D. Wahlsten. Dept. of Psychology, U. of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

Intrauterine position (IUP) effects were studied using fetuses at embryonic day 17.5 from two sublines of the BALB/cWah inbred mouse strain. The midline areas of adult corpora callosa (CC) of about 50% of BALB/cWah1 (line 1) and about 18% of BALB/cWah2 (line 2) mice are very small or non-existent. The prenatal hippocampal commissure (HC) development in BALB is also retarded; however, all but a very few mice do eventually develop an HC of normal midline area. Our index of abnormality for midline commissure development was a standardized (z) score which expressed the number of standard deviations by which the actual CC+HC area differed from area of CC+HC expected according to its body weight. Expected values were derived from data on normal fetuses in the weight range 0.5 to 1.0 g. The results revealed: 1) differences between the lines on mean litter size, body weight, and z score; 2) a right uterine horn advantage on z score in line 2; 3) an ovarian and cervical (vs. middle) position advantage in line 2 for body and placenta weight. Apart from the line 2 right horn effect, no other IUP factor or combination of factors contributed significantly to the variability in degree of CC+HC retardation. A runs test for randomness also provided no evidence for nonrandom placement of fetuses with respect to degree of abnormality.

332.2

STUDY OF TREMOR IN A NEUROLOGICAL MUTANT SUBLINE OF SPRAGUE DAWLEY RATS. E.C. Vega-SaenzdeMiera*, B. Holmgren* and R. Urbá-Holmgren* (SPON: O.C. Ramírez). Departamento de Ciencias Fisiológicas, Instituto de Ciencias. Universidad Autónoma de Puebla, Puebla, Pue., México.

In the Sprague Dawley rat colony of our Department a neurological mutation appeared, characterized by tremor, ataxia , cataplexy and paralysis. The syndrome begins with a fine tremor in the tail and hindlimbs which is visible by simple inspection when rats reach the age of 1 month. Other neurological symptoms appear with increasing age: ataxia at 2-3 months, cataplectic episodes at 6-8 months and hindlimb paralysis after 10 months. This hereditary syndrome is transmitted as an autosomal recessive trait.

The tremor frequency was measured in 8 or more mutant rats at the following ages: 19,21,27,30,45,60,75 and 90 days, using power spectral analysis of the current induced by the movements of a magnet, attached to the animal, on a wire coil, following a procedure similar to that described by Shinozaki(Neurosci. Res. 2:63-76,1984). In about one half of 19-day-old mutants a slight tremor was detectable with a main frequency peak of 13.4 +/- 2.8 Hz. At the age of 21 days all mutants exhibited tremor at an average frequency of 12.1 +/- 1.9 Hz. When 45- day-old a second tremor peak appeared, at 6 +/- 0.2 Hz. In two-month-old mutants, this lower frequency peak (5.7 +/- 0.6 Hz) became the main one, and at the age of three months it was the only tremor peak present (5.4 +/- 0.5 Hz), and had an intentional character.The underlying pathology of this hereditary neurological syndrome is under current study by our group.

Supported by CONACYT grant PCEXNA-050414; SEP grant C87-01-0459 reg DGICSA 861711.

332.3

A DATABASE FOR THE GENETIC ANALYSIS OF GENES OF NEUROLOGICAL INTEREST. M.H. Brilliant and M. Lennon-Pierce*. The Jackson Laboratory, Bar Harbor, ME 04609.

As part of an effort to facilitate the analysis of genes and genetic loci of neurological interest, we have developed a prototype of a highly comprehensive computerized genetic information system. Our database, which deals specifically with neurological mutants, allows us to store, retrieve, sort and analyze mapping data.

A genetic information system - Compendium Genetica (CG) - developed by the Jackson Laboratory's Genetic Information Resource, allows us to compare and position genetic loci across species in relation to total cumulative mapping data. CG contains the history, characteristics, and husbandry, of specific neurological mutants, and has an extensive bibliographic component.

Comparative maps can be superimposed to reveal homologous genetic loci from one mammalian species to the genetic linkage map of another, thus displaying both linkage and synteny conservation.

We present examples of the utility of this information system and in particular, we present the analysis of the locus encoding the gene for tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis.

332.4

RELEVANCE OF DYSTROPHIC CHICKENS AS AN EXPERIMENTAL MODEL FOR HEREDITARY MUSCULAR DYSTROPHY IN HUMANS. D.M. Riedel*, R.K. Entrikin and S.K. Bhattacharya (SPON: R.P. White). Surgical Research Lab., University of Tennessee, Memphis, TN 38163 and University of California, Davis, CA 95616.

Avian muscular dystrophy (MD) in the Davis line 413 dystrophic chickens (DC) has been used for the study of hereditary MD in humans (Muscle & Nerve, 7:130, 1984). Since excessive intracellular Ca accumulation (EICA), abnormal muscle histopathology, and elevated serum CK levels are universally accepted as the earliest detectable pathogenetic changes in Duchenne muscular dystrophy (DMD) in humans (Neurology, 34:1436, 1984) and in BIO-14.6 strain dystrophic hamsters (DH) (Muscle & Nerve, 10:168, 1987), we investigated these variants in 5-week-old and 6-month-old male DC. Muscle [Ca] and [Mg] were determined in the HNO_3 extract by flame atomic absorption techniques (Anal. Lett. 12:1451, 1979). Age and sex matched Davis line 412 normal chickens (NC) served as disease controls.

Unlike DMD and DH, EICA and gross morphological changes with profound cellular necrosis and fatty infiltration were not evident in the myocardium of DC. However, the pectoralis from DC revealed significant EICA accompanied by Mg depletion and classical dystrophic histopathology. Plasma CK activity was also elevated in DC ($p < 0.001$).

We conclude that DC exhibit dystrophic lesions in the skeletal muscle only. On the other hand, DH display both the cardiac and skeletal muscle impairments universally present in DMD. (Supported by NIH grant #AR-38540)

332.5

IMMUNOHISTOCHEMICAL LOCALIZATION OF HPRT IN THE MAMMALIAN BRAIN. H.A. Jinnah,* M.B. Rosenberg, T. Friedmann,* F.H. Gage, (SPON: E. Hess). Depts. of Neurosciences and Pediatrics, UCSD, La Jolla, CA 92093.

Hypoxanthine-guanine phosphoribosyl transferase (HPRT) is an enzyme responsible for converting the bases hypoxanthine and guanine into their respective nucleotides. Deficiency of HPRT in humans results in Lesch-Nyhan syndrome, characterized by several neurological signs including extensor spasticity, choreoathetosis, and self-injurious behavior. In mice, however, absence of HPRT causes no obvious neurological impairments. In view of this difference, it would be interesting to compare the distribution of HPRT in the brains of mice and primates.

HPRT was localized in sections from murine brain using rabbit anti-human HPRT, and visualized by standard immunoperoxidase techniques.

The regional distribution of labelled cells was quite specific; all cells were not uniformly stained. The most intensely stained cells were located in the olfactory tubercle/basal forebrain, inferior colliculus, and deep layers of the superior colliculus. Medium intensity staining was observed in specific cortical layers, amygdala, several hypothalamic nuclei, red nucleus, all brainstem motor nuclei, deep cerebellar nuclei, pontine nuclei, and nucleus of the trapezoid body.

The cellular distribution of label varied according to brain region In some areas, whole neurons were densely stained, with clearly identifiable dendrites. In other regions, staining appeared coarsely granular, not evenly dispersed within the cell cytosol. Granules often appeared clustered at the cell surface, but it remains unclear if they represent intracellular inclusions or exteriorly located synaptic puncta.

We are currently investigating the distribution of HPRT in the brains of human and non-human primates.

332.6

GENETIC MAPPING AND DEVELOPMENTAL EXPRESSION OF GENES ON MOUSE CHROMOSOME 16 IN NORMAL AND ANEUPLOID MICE. B.F. O'Hara*, R.H. Reeves*, C. Bendotti, S. Fisher*, G.T. Capone, J.T. Coyle, M.L. Oster-Granite, and J.D. Gearhart*. Departments of Physiology, Psychiatry, and Neuroscience, Johns Hopkins University Sch. of Med., Baltimore, MD. 21205.

Evolutionary conservation of a number of genes on human chromosome 21 (HSA 21) and mouse chromosome 16 (MMU 16) has led to the study of the trisomy 16 mouse (Ts16) as a model system relevant to studies of human trisomy 21 or Down Syndrome. We have further refined this model by delineating the extent and character of chromosome homology, and by examining the expression of MMU 16 specific genes during development in normal and aneuploid mice. The use of RFLPs and mating schemes involving highly polymorphic mouse subspecies has revealed that gene order has been conserved between the homologous domains of MMU 16 and HSA 21. The proximal to distal gene order on both chromosomes is *App* (Amyloid precursor protein), *Sod-1* (Superoxide dismutase-1), and *Ets-2* (a proto-oncogene sequence). In addition we report that *Gap-43* (Growth associated protein - 43Kd) is distal to *Smst* (preprosomatostatin) on the proximal portion of MMU 16. As both *Gap-43* and *Smst* reside on HSA 3 this confirms and localizes a second syntenic homology on MMU 16. We have examined the expression of genes from the two domains in both Ts16 and Ts19 mice to distinguish gene dosage effects from the generalized effects of aneuploidy *per se*. Quantitative northern blot analysis and *in situ* hybridization methods were used to compare mRNA levels in Ts16 , Ts19, and their respective littermate controls on day 15 of gestation (E15). Since several of these genes may play important roles in normal development, they were examined at other gestational ages. *Smst, Gap-43, App,* and *Ets-2* were all expressed as early as E10-12 (See also G.T. Capone et al., and S. Fisher et al., this meeting; and C. Bendotti et al., European Neuroscience Association meeting, 1988, in press). Each of these genes exhibits a characteristic temporal and spatial pattern of expression, as well as distinct elevations of message in Ts16 mice and variable alterations in Ts19 mice. These studies demonstrate that transcriptional levels of any gene in aneuploid mice cannot be predicted entirely by gene copy number. (Supported in part by HD 19920, HD 19932, HD 22262, and by the Alzheimer's Disease and Related Diseases Foundation).

332.7

EXPRESSION OF THE GENE CODING GROWTH ASSOCIATED PROTEIN-43 (GAP-43) IN THE BRAINS OF NORMAL AND TRISOMIC MICE. G.T. Capone, C. Bendotti, B.F. O'Hara *, M.L. Oster-Granite, J.D. Gearhart *, R. Reeves * and J.T. Coyle. Depts. of Physiology, Psychiatry and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

GAP-43 (F1, B-50, pp46) is a neuronal phosphoprotein associated with axonal elongation during development, with regeneration following injury, and with synaptic modification and plasticity in the adult central and peripheral nervous systems. The levels of expression of GAP-43 increase during fetal and early postnatal life, and then decline, so that adult levels are considerably lower than those found during development in normal animals. O'Hara *et al.* (this meeting) have localized the gene encoding GAP-43 to a region of mouse chromosome 16 (MMU 16) homologous to human chromosome 3.

We have used *in situ* hybridization and Northern blot analysis to determine the developmental expression of GAP-43 mRNA in normal and aneuploid (trisomic) mouse brain. Mice trisomic for MMU 16 (Ts16) were studied to determine the effects of increased gene dosage on the expression of GAP-43 mRNA. Mice trisomic for MMU 19 (Ts19) served as controls for the generalized effects of aneuploidy on development.

As early as day 11.5 of gestation (E11.5) in normal mice, GAP-43 mRNA was detected in whole head preparations; expression increased in the brain approximately 10-fold by postnatal day 1 (PND 1), and continued to increase during the first postnatal week. GAP-43 mRNA can be detected as early as E10 in normal mouse brain by *in situ* hybridization. At E15, GAP-43 mRNA expression was increased 2-3 fold in Ts16 mouse brain, relative to euploid littermate controls, but was decreased slightly (20%) in Ts19 mouse brain, relative to their euploid littermate controls. Alteration in the expression of GAP-43 mRNA observed in these aneuploid mice may contribute to some of their CNS developmental abnormalities.

332.8

DEVELOPMENTAL EXPRESSION OF AMYLOID PRECURSOR PROTEIN IN NORMAL AND TRISOMY 16 MICE: RELEVANCE TO DOWN SYNDROME AND ALZHEIMER'S DISEASE. S. Fisher*, R.A. Morgan*, B.F. O'Hara*, J.D. Gearhart*, M.L. Oster-Granite. Developmental Genetics Lab, Johns Hopkins School of Medicine, Baltimore, MD 21205

The A4 peptide is a major component of the cerebrovascular amyloid plaques and extraneuronal senile plaques found in the brains of Alzheimer's disease patients and aged Down syndrome (DS) individuals. The gene encoding the amyloid precursor protein (APP) maps to human chromosome 21, and its homolog to mouse chromosome 16. We have cloned a full-length mouse APP cDNA, which has a 97% homology with human APP at the amino acid level. This high homology and the widespread distribution of the mRNA suggest a conserved function for APP. To better understand its function and whether its early overexpression contributes to the developmental abnormalities seen in DS, we studied its expression in normal and trisomy 16 (Ts16) mice. Northern blots of tissues from different pre- and postnatal stages were hybridized with probes from mouse APP cDNA or end-labeled synthetic oligonucleotides corresponding to the forms of APP generated by differential splicing. Western blots were done on tissues from the same ages with antibodies raised to synthetic peptides from portions of the APP sequence. APP expression was detected at gestational day 10, and attained adult levels by gestational day 13. Relative expression of the form of APP with the protease inhibitor insert was higher in non-neural tissues. At all gestational ages examined, APP expression was higher in Ts16 mice. The Western blots showed greater levels of APP in non-neural tissues than predicted by the mRNA levels; this suggests a difference in stability between the different forms, leading to a greater turn-over and lower steady state levels for the form without the insert. The early overexpression of APP in the Ts16 mouse may enable us to study the contribution of APP overexpression to the developmental abnormalities seen in DS.

332.9

OBSERVATIONS ON THE CNS OF LONGER LIVED MYELIN DEFICIENT RATS. K.F. Jackson*, I.D. Duncan, M.R. Wells and S.F. Worth*. School of Vet. Med., U. of Wisconsin-Madison, WI 53706 and VA Medical Center, Washington, D.C. 20042.

Myelin deficient (md) rats have rarely survived beyond 35 days of age. Recently, certain female carriers of this X-linked dysmyelinating disorder, derived from the original Wistar breeding stock, have produced affected male pups which survive for up to 60 days. One each of these mutants at 40 and 50 days, and 2 at 60 days of age, were perfused with aldehyde fixatives and the CNSs processed for light (LM) and electron microscopy (EM). On LM there was an apparent increase in the number of myelinated axons on the ventral columns of the spinal cords of these older md rats, compared to mutants of 30 days of age. There was an apparent decrease in the total glial cell count in the intracranial optic nerve and spinal cord. There were estimated to be only 140 cells per optic nerve section at 60 days, compared to 230 glial cells per section observed in 30 days old md rats. EM examination suggested that many of the cells were astrocytes, although there was no obvious astrogliosis in either the cord or optic nerves. Undifferentiated cells and microglia were also present in both regions, as well as some abnormal oligodendrocytes, containing distended rough ER. These observations suggest a continued decrease in the glial cell population in older md rats, with particular loss of oligodendrocytes. However, a number of these cells must be viable in the older mutants, as myelin sheaths are maintained, and even appear to increase in the spinal cord. (Supported by NIH grant NS23124 and NMSS grant RG1791).

332.10

MOLECULAR ANALYSIS OF THE SHAKING PUP MUTATION. N. Nadon*, I.D. Duncan, L. Hudson* (SPON: M. Behan). National Institutes of Health, NINCDS, Bethesda, MD 20892 and School of Vet. Med., U. of Wisconsin, Madison, WI 53706

The shaking pup is a sex-linked dysmyelinating mutant of the dog in which the myelin proteolipid protein (PLP) is reduced greater than 100-fold, while DM-20, the alternatively spliced form of the protein, is reduced only about 6-fold (Yanagisawa et al., J. Neurochem. 49:1812-1917, 1987). In order to identify the molecular defect, DNA and RNA from shaking pups was compared to that from normal littermates. Northern blot and slot blot analysis showed that the PLP mRNA, a single band of 3.2 kb, is detectable in the brain and spinal cord of both normal dogs and shaking pups by two weeks of age. The PLP mRNA is the same size in the shaking pup as in the normal dog, but is reduced to about 10% of normal levels. Southern blot analysis indicates that there are no gross deletions or rearrangements of the PLP locus in the shaking pup. Genomic DNA libraries from normal dog and shaking pup were constructed in the EMBL3 vector and screened with a human PLP cDNA. The PLP clones were mapped and sequence analysis is underway to locate the defect in the shaking pup PLP locus. (Supported by grants from the NIH (NS23124) and NMSS (RG1791) to IDD).

332.11

FREQUENCY ANALYSIS OF WHOLE BODY OSCILLATION IN SHIVERER (SHI) MOUSE. R. S. Pozos, P. Iaizzo*, and C. vonRabenau*. Univ. of Minn., Duluth, School of Medicine, Duluth, MN 55812.

Mice that are homozygous for the autosomal recessive mutation which has been called shiverer (shi) lack myelin basic protein and exhibit a range of behavior patterns, including tremors, convulsions, and death. The tremor has been called a "shiver" but no analysis of the electromyogram or motion has been reported. Wire electrodes were placed in both hind limbs of shiverer (shi) mice* and an accelerometer was placed securely on their back. The animals were allowed to walk freely and the EMGs and motion were monitored during rest as well as motion. Various 16 second portions of the EMG and motion record were analyzed using traditional spectral and cross-spectral techniques on a VAX-station II computer. Analysis indicated that there were distinct bursts of EMG activity and motion at a frequency of 12 Hz. The EMG bursts were synchronous in the hind limbs and were present at rest as well as during motion. This 12 Hz oscillation in these genetically demyelinated mice did not resemble human shivering in that it did not stop during motion nor was it abated with a rise in ambient temperature.

*Donated by Dr. H. David Shine, Center for Biotechnology, Baylor College of Medicine.

332.12

OLIGOSACCHARIDES AND ABNORMAL NEURULATION IN THE DELAYED SPLOTCH MUTANT. R.G. Higbee, J.L Fiacco,* T. Vanden Hoek,* W. Goossens,* D.G. McLone, and P.A. Knepper. Division of Neurosurgery, Children's Memorial Hospital and Northwestern University Medical School, Chicago, IL 60614.

Glycoconjugates play major roles in many cellular functions, e.g., cell migration and adherence, which are involved in neurulation. Previous studies by our laboratory have indicated correlations in the time of appearance, type and distribution of oligosaccharides with normal as well as with teratogen-induced (vitamin A) abnormal neurulation which suggest that oligosaccharides may serve as molecular participants in the process of neurulation. In the present study, a genetic model of abnormal neural tube closure, the delayed splotch mouse mutant (Sp/Sp), was studied on gestation days 12, 14, and 16 by a battery of FITC-labeled lectins using low-light intensity video-microscopy and by analysis of micro-dissected neuroepithelium (NE) using PAGE, western blots, and peroxidase-coupled lectins to detect carbohydrate moieties on specific proteins. The gestation day 16 Sp/Sp embryos exhibited varying degrees of myeloschisis of the sacral region, and concomitant changes were observed in the staining patterns of several FITC-labeled lectins. Image analysis of FITC-WGA binding by control NE in 1-um plastic sections exhibited spatial and temporal changes during neurulation, particularly in the zone of closure; FITC-WGA binding was altered in the NE of Sp/Sp embryos. Western blots demonstrated a decrease in the WGA-binding of 15-Kd and 80-Kd proteins in both the caudal and rostral NE in gestation day 16 embryos with myeloschisis compared to littermates. The protein profiles of Sp/Sp, littermates, and control embryos were similar on silver-stained gels, i.e., no changes were observed in the 15-Kd and 80-Kd proteins. The results of this study demonstrated changes in oligosaccharides in Sp/Sp embryos; these changes were similar to the abnormalities of the splotch mutant and vitamin A-induced myeloschisis in the mouse. Thus, these results indicate that oligosaccharides may serve as molecular participants in the events of abnormal neurulation and provide additional evidence for the importance of glycoconjugates and the process of neurulation.

332.13

NEW MOUSE MUTANT WITH KINKY TAIL AND SEVERE CORPUS CALLOSUM (CC) DEFECT. B. Cassells and J. V. Clifton*. Dept. of Psychology, Univ. of Waterloo, Waterloo, ON, N2L 1V8 CANADA.

A recessive incompletely penetrant mutation affecting tail and CC morphology has been detected in BALB/c mice obtained from CRBL and the gene name kc (kinky tail, CC defect enhancer) proposed. At or < 2 wks after birth, mutants show variably shortened, kinked, twisted tails. Tail length at weaning (10-90% of normal) is inversely related to kink detection age. Pre- but not post-weaning growth and survival are reduced. Female but not male mutants breed.

All BALB/c kc/kc detected at birth have mid-sagittal CC areas < 30% normal. In contrast, only a minority of kc/kc late-onset, kc/+, +/?, and +/+ BALB/c's of this line show CC defects, consistent with reports on other BALB/c stocks. The effect of kc/kc on the CC depends on its interaction with other recessive elements of BALB/c heredity. This combination of characters, though sharing some features with previously described mutations, appears to be unique and indicative of a new recessive mutation. This mutant may be useful to those studying the heredity and embryology of callosal development or behavioral and physiological functions of the CC.

332.14

AUTOMATED ANALYSIS OF CIRCADIAN BEHAVIOR AND EEG POWER SPECTRA OF ALCOHOL-PREFERRING AND -NONPREFERRING RATS. S. Morzorati, B. Lamishaw*, L. Lumeng*, T.-K. Li* and J. Clemens, Dept. Med. and Regenstrief Inst., Ind. Univ. Sch. Med. 46223 Eli Lilly & Co. 46285, Indianapolis, IN.

Low dose ethanol has been shown to produce a differential response in the behavior and EEG of rats selectively bred for alcohol-preference (P) and -nonpreference (NP). Whether these rats differ in baseline circadian behavior and EEG is the focus of the present study.

The frontocortical and hippocampal EEG and movement of P and NP rats were recorded for 24 hours. Eight-second epochs of EEG taken once every minute were subjected to power spectral analysis. Behavioral maps were constructed for each rat. Time spent in non-REM sleep, REM sleep, awake/immobile, and moving and the corresponding power spectra were compared across the two rat lines in the light and dark. While P and NP rats did not differ significantly in total time spent in any behavior, P rats did show more prolonged periods of activity in the dark. Following lights on, the latency to the first sleep cycle was markedly shorter in P than in NP rats. P rats had more power in the lower frequencies during non-REM sleep, suggesting a deeper sleep. Thus, while P and NP rats regulate sleep according to a homeostatic mechanism, they differ in their latency to and depth of sleep perhaps because of differences in activity duration during the dark. (Supported by PHS AA 03243.)

332.15

FRONTAL LOBE MATURATION IN DOWN SYNDROME: IMMUNOCYTOCHEMICAL AND STRUCTURAL STUDY OF THE CHOLINERGIC SYSTEM.
I. Kostović, A. Bunarević, L.B. Hersh, G. Bruce, A. Fučić, B. Šain. Sect. Neuroanatomy, Zagreb Univ. Sch. of Med., 41001 Zagreb, Yugoslavia and Dept. Biochemistry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX 75235.

The aim of this study was to determine whether abnormalities of the cholinergic basal forebrain-cortex system in Down syndrome (DS) occur during the infancy and early childhood. We applied ChAT-immunocytochemistry (anti-human placental ChAT antibody of Hersh-Bruce), AChE-histochemistry and Nissl method, analyzing basal forebrain (BF) and frontal cortex on postmortem tissue obtained from 5 individuals with DS and age-matched controls (newborn-2,5 years). In newborn, more than 80% of BF neurons are ChAT-reactive in both DS and controls; ChAT-reactivity was enhanced in DS basal forebrain. Cortical cytoarchitectonics and AChE-patterns appear to be normal. In older group (2-2,5 years) AChE-reactive pyramidal neurons were not found but fibre staining was enhanced. Layer III pyramidal neurons showed the vacuolar degeneration. In both age groups the mean perikaryal size was not different in comparison with age-matched controls. In conclusion, basal forebrain-cortical system appears structurally normal in the newborn, with possibly increased ChAT-synthesis. At 2,5 years first abnormalities appear in cortical associative layers, indicating that cortical pathology may be a primary phenomenon. Supp. by Joint-Board No. 698 and SIZ za znanost SRH.

332.16

NOREPINEPHRINE AND MET-ENKEPHALIN CONCENTRATIONS IN SPECIFIC BRAINSTEM AND SPINAL CORD REGIONS OF THE GENETICALLY EPILEPTIC, TOTTERING (tg/tg) MOUSE. L.C. Abbott, B. Weber*, and R.H. Abhold. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164-6520.

Elevated levels of norepinephrine (NE), mainly in cortical regions of the brain, have been reported to be responsible for petit mal and focal motor seizure activity in tg/tg mice. However, thorough analysis of NE levels in the brainstem and spinal cord have not been previously done. Since these CNS regions may also be important in the generation and/or maintenance of these forms of seizures, NE levels were measured via HPLC-EC in several brainstem and spinal cord regions in adult tg/tg mice relative to control (+/+) mice. Decreased NE was observed in the pons from tg/tg mice while other brainstem and spinal cord regions exhibited increased NE levels.

Methionine-enkephalin (MET-ENK), known to colocalize with and modulate the actions of NE, was also measured via RIA in the brainstem. In those areas NE was decreased in tg/tg mice, MET-ENK also was decreased, relative to +/+ mice. These data raise the possibility that decreased NE and MET-ENK may be involved in epileptogenesis and/or maintenance of seizure activity in the tottering mouse. Measurements of MET-ENK levels in spinal cord regions are in progress. Supported by NIH grants RR00515 and RR05-46525.

SUBCORTICAL VISUAL PATHWAYS III

333.1

PATTERNS OF RETINAL TERMINATIONS IN RODENT SUPERIOR COLLICULUS LABELED WITH HRP AND WGA-HRP TRACERS. S. Agarwala, J.G. May III & H.M. Petry. Dept. of Psychology, S.U.N.Y. at Stony Brook, NY 11794

Retinal projections were examined after monocular intravitreal injections of either horseradish peroxidase (HRP, 50 ul of a 30% solution) or wheat germ agglutinin conjugated HRP (WGA-HRP, 30 ul of 1% solution) in ground squirrels (Citellus tridecemlineatus) and rats (Rattus rattus). Following various survival schedules, brains were processed for TMB histochemistry.

The retinal projection patterns seen after injection of either HRP or WGA-HRP were similar for all structures except for the contralateral superior colliculus (SC). In ground squirrel, this projection exhibited a discontinuous pattern of vertical slabs throughout the superficial layers in all HRP cases and in some WGA-HRP cases. However, uniform labeling of the contralateral SC occured in other WGA-HRP cases. These tracers also produced similar differences in the pattern of label in the rat SC. The differences could not be attributed to obvious methodological factors, nor could they be adequately explained by superior WGA-HRP sensitivity or its transneuronal transport. The similarity of labeling in all other visual projections regardless of tracer, makes the differences in the SC projection very intriguing. (Supported by NSF grant BNS 8519623 to JGM and NIH grant R29-EY07113 to HMP).

333.2

EFFECT OF LESIONS INVOLVING THE PARABIGEMINAL NUCLEI ON CHOLINE ACETYLTRANSFERASE AND ACETYLCHOLINESTERASE ACTIVITIES IN RAT SUPERIOR COLLICULUS. C.D. Ross[1], W.B. Farms*[1], J.D. Dunn[2] and D.A. Godfrey[1]. Depts. of Physiology[1] and Anatomy[2], Oral Roberts University, Tulsa, OK 74171

Activities of choline acetyltransferase (ChAT), catalyzing the synthesis of acetylcholine (ACh), and acetylcholinesterase (AChE), catalyzing the destruction of ACh, are high in the superior colliculus (SC), including the superficial layers that receive substantial projections from the parabigeminal (PBG) nuclei. Activities of ChAT and AChE were assayed in samples microdissected from superior collicular layers in rats killed one week after electrolytic lesions were made to areas including the PBG. Lesions destroying PBG bilaterally produced a 60-80% decrease in ChAT activity in superficial SC layers, with a slight (about 20%) decrease in some deeper layers, but no significant change in AChE activity. Lesions of similar size, but rostral to and sparing the PBG, produced no change in activity of either enzyme. These results indicate that a substantial cholinergic projection to the superficial SC layers originates in the PBG. ChAT activity in the SC is not associated with the optic projection, since it does not decrease following enucleation. Therefore, ChAT activity remaining after PBG destruction could be related to a small projection from another source or to intrinsic cholinergic neurons in the SC. (Supported by NIH grant EY-03838)

333.3

ORIENTATION OF THE DENDRITIC ARBORS OF HORIZONTAL CELLS IN THE HAMSTER'S SUPERIOR COLLICULUS. S. Ruiz*, W.H. Rohrer, M.M. Nikoletseas, R.D. Mooney and R.W. Rhoades (SPON: S.E. Fish). Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Horizontal (H) neurons are a prominent cell type in the superficial layers of the rodent SC. The orientation of the dendritic arbors of these neurons has been the subject of some controversy. Based upon examination of Golgi material, Valverde (Z. Anat. Enwickl. Gesch., 142:117, 1973) concluded that the dendritic arbors of H cells in the mouse paralleled the frontal plane. Langer and Lund (J. Comp. Neurol., 158:405, 1974) suggested that the orientation of H cell dendrites paralleled isoazimuth and isoelevation lines of the visual field representation in the rat's SC. Tokunaga and Otani (Exp. Neurol., 52:189, 1976) concluded that H cells in rat have no preferred dendritic orientation. We used computer-aided reconstruction of horseradish peroxidase-filled neurons to determine whether H cells in hamster have a preferred dendritic orientation. In 9 of 10 H cells, the mediolateral extent of the dendritic tree exceeded its rostrocaudal dimension. The average ratio of these two dimensions was 2.1 (sd=1.2). The long axis of H cell dendritic arbors was oriented at an average angle of 20.1° (sd=13.2) to the frontal plane. Thus in hamster, H cells do have a preferred dendritic orientation and it is generally parallel to the frontal plane. Supported by BNS 85-00142, EY 04170 and NS 07229.

333.4

INTERLAMINAR PROJECTIONS IN THE HAMSTER'S SUPERIOR COLLICULUS. R.D. Mooney, S.E. Fish, M.M. Nikoletseas, W.H. Rohrer and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Marshall University Medical School, Huntington, W VA 25700.

We used Phaseolus vulgaris leuco-agglutinin to trace interlaminar projections within the superior colliculus (SC) of normal hamsters. The superficial SC laminae projected to all of the deep layers and also to the periaqueductal gray. This projection terminated most heavily in the stratum griseum intermediale and stratum griseum profundum and it was topographically organized. There was also a projection from the deep to the superficial laminae, but it was relatively sparse. The trajectory of the superficial to deep projection was angled laterally from a line that extended orthogonally to the SC surface from the injection site center. We assumed such orthogonals to be the most likely trajectories of SC projection lines, i.e. lines connecting the centers of receptive fields that have constant elevation and azimuth in the visual field. Because of this apparent mismatch, we combined PHA-L tracing with receptive field mapping to determine how well the trajectory of the SC-interlaminar pathway matched actual projection lines in the superficial and deep layers. As was the case for the trajectory of the SC-interlaminar projection, visual projection lines in the hamster's SC were angled laterally from lines orthogonal to the SC surface. Supported by BNS 85 00142, EY 04170 and NS 077229.

333.5

PRESYNAPTIC TRANSFER PROPERTIES OF THE RETINOTECTAL CONDUCTION GROUPS IN THE SUPERIOR COLLICULUS BRACHIUM OF THE HOODED RAT. D.Impelman and D.Fox. Dept. of Physiol.,UTHSC, S.A.,TX AND C.of OPT.,UH, Houston, TX.

Previous electrophysiological studies have shown that the fast conducting t1/Y-like(12.5m/s) and the middle conducting t2/x-like(6.0m/s) axons branch to innervate both the superior colliculus and the dLGN(Vis.Res.8:867). HRP retinotectal studies have demonstrated that the major axonal projection to SC(63%) is comprised of the slow conducting(3.5m/s) t3/W-like axons(Z.Mikrosk.Anat.Forsch.92: 283). We are interested in the presynaptic transfer properties of retinotectal axons and have studied their functional characteristics in OT and SC brachium depth recordings. The data show that 1)the relative amplitude measurements of t1, t2 and t3 are better correlated with SC HRP estimates of the RGC conduction group projections than with unit latency histograms; 2)orthodromic t3 conduction is preferentially slowed(15-20%) in brachium recordings; 3)t2 and t3 brachium recovery functions are depressed with a timecourse which parallels the SC postsynaptic field potential response. The t3 response depression is sensitive to the 4AP K+ channel blocker suggesting K+ accumulation may contribute to the SC field potential postsynaptic depression. Supported by NIEHS Grant 03183(DAF).

333.6

LIGHT-EVOKED RESPONSES FROM SINGLE MORPHOLOGICALLY IDENTIFIED TECTAL CELLS IN THE GOLDFISH. D. M. Guthrie* and S. C. Sharma. Department of Ophthalmology, New York Medical College, Valhalla, NY 10595.

91 intracellular penetrations were made into tectal neurons of 26 adult goldfish using HRP filled microelectrodes. Resting potentials varied between 30-70mvs; action potentials ranged from 10-45mvs. A 5ms flash provided visual stimulus. Following iontophoretic injections, 36 sites were identified in histology sections. 21 identified unit injections corresponded to the following morphological types described by Meek and Schellart (1978): 4 large diameter afferent fibres; 8 type I cells (pyramids); 1 type III cell; 1 type VI cell; 7 cells with somas in the SPV - 4 type XIV and 1 type XV cell and 2 glia cells. Afferent fibers provided 'on' responses, either phasic, tonic or bursting without spontaneous background; type I cells mostly yielded 'on' dominated responses, with weak or negligible 'off' components. The spontaneous activity consisted of regular bursts. One cell provided only a weak 'off' response, and one cell did not respond to light flash. Type III cell provided delayed 'on' response, while the type VI cell produced an irregular response suggesting equally strong 'on' and 'off' components. Responses from SPV cells were very variable. Glial cells appeared silent, but type XIV and XV cells were either silent or spontaneously active at a low or high level. Responses were phasic 'on'/phasic (delayed) 'off' or tonic 'on'-'off'.
Supported by NEI 01426.

333.7

AMPHETAMINE INCREASES RECEPTIVE FIELD SIZE IN THE SUPERFICIAL LAYERS OF CAT COLLICULUS. K.L. Grasse[1], R.M. Douglas [2], and J.R. Mendelson [3]. [1]Dept of Psychology, York Univ., North York, Ontario, [2]Dept of Ophthalmology, Univ. of British Columbia, Vancouver, B.C., and [3]Dept of Physiology, Univ. of Toronto, Toronto, Ontario, Canada.

Visual response properties were examined in the superficial layers of the superior colliculus (SC) of anesthetized, paralyzed cats before and after i.v. administration of dextroamphetamine sulphate. Units were tested (through the contralateral eye only) for a number of visual response properties: receptive field (RF) size and position, direction selectivity, strength of inhibitory surround, and the incidence of "on" or "off" responses. 15 minutes to 1 hour after amphetamine injections (2ml of 10mg/ml), RF size began to gradually increase. RFs expanded by some 2-10 times (mean 4.5) over the following 1-4 hours. Subsequently (i.e., 4-7 hours post-injection), RF size returned to pre-injection dimensions. In most cases, RF size did not increase equally in all directions, but rather displayed asymmetrical patterns of expansion. In addition to changes in RF size: 1) responses to flashed stimuli became much more vigorous; 2) surround inhibition, when present, usually became much weaker; and 3) no consistent effects were noted in direction selectivity. It is possible that the elevated activity levels in the SC resulting from increases in RF size, will affect the peak velocity and/or timing of visually evoked saccades.

333.8

CONTRIBUTIONS OF THE SUPERIOR COLLICULUS OF THE MONKEY TO VISUAL SPATIAL ATTENTION. Caroline Kertzman and David Lee Robinson Laboratory of Sensorimotor Research, National Eye Institute, N.I.H., Bethesda, MD 20892.

Visual spatial attention refers to the ability to select images for special use independent of eye movements. We have trained rhesus monkeys on a task developed by Posner: the animal fixates a spot of light and contacts a bar when target lights appear. Reaction times are faster for validly cued targets than for invalidly cued ones. When we injected muscimol into the superior colliculus, the animal was slow in responding to validly cued targets in the visual field contralateral to the injection. Reaction times were substantially delayed for the same target when it was preceded by a cue in the other field. The animal behaved as if it had difficulty with visual spatial attention. In control monkeys, neurons in the superficial layers of the superior colliculus were easily driven in this task. Cells in the foveal representation responded when the fixation target was in the neuron's receptive field; there was no change in activity associated with the cue (which is hypothesized to shift attention). When the cue and/or target were positioned in the visual receptive field of a neuron, cells were driven briskly. Disregarding simple refractory effects, there were no consistent and significant changes in the response of a cell to a target dependent on cue validity. The effects of pharmacological manipulation of the superior colliculus, however, show that this structure contributes substantially to visual spatial attention. These data show that the tectum has a visual function which is independent of eye movements.

333.9

MULTIPLE VISUAL MAPS IN DEEP SUPERIOR COLLICULUS OF CATS. M. A. Meredith and B. E. Stein, Depts. Anat. and Physiol. Medical College of Virginia/VCU, Richmond, VA 23298.

Topographic register among sensory representations is thought to be a critical factor in facilitating coordinated responses to a variety of sensory cues. This sort of multisensory registry has been demonstrated in the superior colliculus (SC) by relating the superficial layer retinal map to the deep laminae maps of the body and auditory space. Yet, because of the differing afferent and efferent connections of these laminae and their differing behavioral roles, this superficial-deep layer sensory register may be of little functional significance. In the present experiments, the deep laminae visual representation was examined directly. Unimodal visual neurons (n=52) had receptive fields (RFs) of intermediate size with nasal-temporal borders varying with AP and elevation varying with ML position in SC. In contrast, visual neurons that received auditory inputs (n=76) had significantly larger RFs whose nasal-temporal borders varied with AP but whose elevation was generally insensitive to different ML locations. These data demonstrate the sharing of axes by visual and nonvisual representations in the deep laminae. In addition, the differing patterns of visual RF distribution among unimodal and multisensory neurons, coupled with their different efferent projections, suggest that multiple, and functionally distinct visual maps coexist in the deep laminae. Supported by NS-22543.

333.10

SPATIAL CHARACTERISTICS OF MULTISENSORY INTEGRATION IN BEHAVING CATS. Lawrence McDade*, M. Alex Meredith and Barry E. Stein (SPON: Karl C. Corley). Depts. of Physiology and Anatomy. Medical College of Virginia/VCU. Richmond, Virginia 23298

Our previous studies have shown that a simple spatial rule characterizes multisensory integration at the cellular (superior colliculus) and behavioral levels: coincident multisensory cues produce enhancement whereas disparate stimuli produce depression (or no effect). In the present studies, we evaluated the resolution of this system behaviorally by determining the spatial separation between stimuli necessary to convert enhancement to depression. Three cats were trained to attend, orient and approach a low intensity visual stimulus at various positions along a semicircular track. A low intensity coincident auditory stimulus enhanced correct responses whereas the same stimulus inhibited correct responses when located as little as 15 degrees medial to the visual. When the auditory stimulus was 15-60 degrees lateral to the visual, it not only failed to depress correct responses but often enhanced them. This medial-lateral difference modifies the spatial rule as applied to behavior and may be due to the large excitatory receptive fields in multisensory neurons which allow stimulus position to vary widely in the periphery and still produce excitation in the same auditory-visual cells. Supported by NIH Grant NS 22543.

333.11

INTRACELLULAR HRP STUDY OF VISUAL AND INFRARED TERMINAL AND POSTSYNAPTIC CELLS IN THE OPTIC TECTUM OF THE RATTLESNAKE CROTALUS VIRIDIS. P. H. Hartline, R. V. Stirling, and D. Berson. Eye Research Institute, Boston, MA and Brown University, Providence, R.I.

The infrared sensitive pit organ of crotaline snakes sends spatial information to the optic tectum via a specialization of the trigeminal pathway. We are interested in identifying tectal cells that integrate visual information and infrared (IR) information from the pit as a step towards describing the infrastructure of multisensory integration.

We have recorded intracellulary from visual and IR terminals and postsynaptic cells in the tectum of anaesthetized snakes using bevelled HRP-filled microelectrodes. After qualitative identification of each unit's response properties to IR and visual stimuli, HRP was ejected. Filled processes were reconstructed from serial sections after DAB processing (Adams, 1981).

The infrared afferents (from nucelus reticularis caloris in the brain stem) are large diameter fibers arborizing extensively (~500 um ant/post by 500-1000 um med/lat) in the stratum griseum centrale (SGC); smaller sizes were found rostrally. This arbor size does not seem to be reflected by IR receptive field sizes of postsynaptic cells. Terminal arbors of the much finer retinal fibers are smaller and are found above SGC in stratum fibrosum et griseum superficiale.

OR neurons, which responded to both visual and IR inputs had dendrites that arborized in both upper visual and deeper IR input layers of the tectum; several different dendritic architectures were noted. Most of these were tectal output cells with crossed or uncrossed descending axons. We recovered a few neurons responding primarily to visual or IR input whose responses were modulated (enhanced or depressed) by concurrent stimulation in the other modality; these cells arborized in both superficial and deeper tectal layers. Unimodal visual or IR cells were rarely encountered, probably because of their small size.

333.12

VISUALLY ELICITED AVOIDANCE IN THE MONGOLIAN GERBIL. C. G. Ellard. Department of Psychology, Queen's University Kingston, Ontario, CANADA.

The trajectory of escape from overhead visual stimulation is poorly predicted by the trajectory of the approaching threat (Ellard and Goodale, in press). Escape responses are usually directed toward a shelter, when one is available. In the present experiment, the power of stimulus context to modify escape responses was tested by requiring animals to run toward a threat in order to reach shelter. Animals were placed in a rectangular arena for five minutes. Time sampling methods were used to record the animals' behavior during this period. Following this, an overhead visual stimulus was presented. The immediate response to the stimulus was noted and behavior was sampled for a further five minutes. Under these conditions, escape responses were directed toward the shelter on only about half the trials and only when the animal was close to the shelter at stimulus onset. This finding distinguishes escape behavior from simple "startle". Lesions of the uncrossed descending tectal efferents abolish visually elicited escape whether shelter is available or not (Ellard and Goodale, in press). The present results suggest that this pathway might have access to information regarding the location of shelter. Alternatively, tectal output may serve as a "trigger" while the details of the response are determined by modulatory influences from other brain areas.

NEURAL PLASTICITY IN ADULT ANIMALS: INDUCED EFFECTS II

334.1

THERMAL DEPENDENCE OF NEURAL ACTIVITY IN THE HAMSTER AND RAT HIPPOCAMPAL SLICE M. P. Thomas*, M. S. Krelstein*, and J. M. Horowitz. Dept. of Animal Physiology, Univ. of Calif., Davis, CA 19616.

Previous studies have shown that the temperature just below that at which a population spike could be recorded, T_t, was 4 degrees lower in a hibernator, the chipmunk, than in a nonhibernator, the rat (Hooper et al, J. therm. Biol. 10:35, 1985). In addition to this study showing phylogenetic adapation, in a study on acclimation to cold (Thomas et al. J. therm. Biol. 11:213, 1986) showed that T_t for hibernating hamsters was 12.3 $\pm$ 0.3°C (mean $\pm$ SEM) compared with the higher T_t of 15.8 $\pm$ 0.9°C for noncold-acclimated hamsters. In this study the amplitudes of population spikes evoked by Schaffer collateral stimulation (using methods described in papers cited) were measured in noncold-acclimated hamsters and rats as bath temperature was lowered from 35°C to T_t. As temperature decreased the amplitude increased to a maximum and then decreased in both hamsters and rats. Long-term potentiation (LTP) was evoked above 22°C by pulse train stimulation (15 trains at a rate of 3.33 Hz where each train was comprised of 4 shocks with an intershock interval of 10 msec). These results suggest that above 22°C the temperature effects on selected cellular mechanisms in CA1 pyramidal cells evoked by Schaffer collateral stimulation do not markedly differ in the hamster (a hibernator) and rat (a nonhibernator). Supported by NASA grant NAG 2-341.

334.2

A COMPUTER ALGORITHM FOR SEPARATING OVERLAPPING SYNAPTIC CONDUCTANCES. **Wei Su*, G.J. Pacelli* and S.R. Kelso. Dept. of Biological Sciences and Dept. of Electrical Engineering, University of Illinois at Chicago, Chicago, IL 60680.**

The activation of an afferent synaptic input to area CA1 of the hippocampus normally results in the activation of more than one type of synapse onto a single postsynaptic neuron. The resultant composite synaptic waveform comprises contributions from each conductance which are added together, or integrated in a manner determined by the electrotonic structure of the postsynaptic cell. Various experimental, pharmacological or pathological conditions can result in changes in the shape of the synaptic waveform. In such cases it is desirable to be able to determine which of the component synaptic conductances has changed. Therefore, making certain assumptions, we have begun to develop a set of computer algorithms for unambiguously breaking a composite synaptic waveform into its individual components.

We first constructed a series of waveforms composed of two alpha functions that approximated a hippocampal EPSP and IPSP and overlapped with a short delay (Brown and Johnston, J. Neurophys. 50:487, 1983). The composite waveform was thus defined by five parameters: $Alpha_{EPSP}$, K_{EPSP}, $Alpha_{IPSP}$, K_{IPSP}, and Tau. Computer algorithms were then developed which utilized an optimal search technique to decompose these waveforms and to approximate the original parameters. Finally, the algorithms were used to fit synaptic current waveforms obtained using single electrode voltage-clamp recordings in hippocampal neurons.

Supported in part by NIH grant NS24591.

334.3

A BIOLOGICALLY PLAUSIBLE IMPLEMENTATION OF A HEBBIAN COVARIANCE ALGORITHM. E.W. Kairiss, C. L. Keenan and T. H. Brown. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

A Hebbian learning mechanism is one in which synaptic modification is governed by the conjunction or covariance between pre- and postsynaptic activity. Sejnowski (*J. Theor. Biol.*, 69:385, 1977) noted that a Hebbian conjunctive mechanism that *only* produces permanent synaptic enhancement will encounter the problem of runaway instability, due to spurious coincidences, even when pre- and postsynaptic activities are uncorrelated. This runaway instability can be avoided by assuming rapid passive decay of the synaptic enhancement.

But what about more durable information storage? A Hebbian covariance mechanism can avoid the instability problem without incorporating rapid passive decay of the synaptic modifications (Sejnowski, *ibid*). Positive correlations produce synaptic enhancement and negative correlations produce synaptic depression. A covariance-type of synaptic mechanism may account for aspects of the normal self-organization of the visual system (Linsker, R., *Proc. Nat. Acad. Sci. USA*, 83:7508, 1986) as well as certain disruptive effects of monocular occlusion (Wiesel, T.N. and Hubel, D.H., *J. Neurophysiol.*, 26:1003, 1963; Reiter, H.O. and Stryker, M.P., *Proc. Nat. Acad. Sci. USA*, in press) on visual cortical development (Brown, T.H. *et al*, *Ann. Rev. Neurosci.*, submitted).

Our simulations have suggested ways to construct, from known neurobiological phenomena, synaptic computations that share some of the desirable features of a covariance mechanism. One solution is just a sum of three identified types of synaptic plasticity--a conjunctive type of synaptic enhancement and two types of synaptic depression. This three-process synaptic mechanism avoids the instability problem and offers a biologically plausible synaptic substrate for certain of the developmental phenomena cited above (Brown, T.H. *et al, ibid).* Our results immediately raise the question as to whether these three forms of plasticity actually co-occur in the same synapses or local circuits. *(Supported by the MRC and AFOSR)*

334.4

LOW-FREQUENCY SYNAPTIC DEPRESSION IN THE IN VITRO DENTATE GYRUS FOLLOWING PICROTOXIN BLOCKADE OF GABA MEDIATED INHIBITION. P.C. Rinaldi and T.A. Leach*. Dept. of Surgery, Div. of Neurosurgery, Univ. of California at Irvine, Irvine, CA 92717.

Low-frequency synaptic depression (LFD) and habituation may play an important role in the modulation of the flow of information through adaptive neural networks. The hippocampal formation, specifically the perforant input to dentate granule cells, provides an excellent system in which to investigate LFD in mammalian brain. As part of on-going efforts to investigate LFD at this synapse, experiments have been conducted employing the GABA blocker picrotoxin to aid in determining the influence that GABA mediated inhibition may have on LFD in this network. Field potentials reflecting population spike or dendritic responses were observed during LFD and compared to the same measure obtained from the same tissue following exposure to picrotoxin.

Hippocampal slices from rats were conventionally prepared and maintained. Responses to bipolar electrode stimulation of the perforant path inputs were recorded with micropipettes and evaluated on-line. The rate of stimulation during control and recovery periods was 1 every 30 seconds; during the intervening period for LFD it was 1 Hz.

Following picrotoxin exposure of 40 to 80 minutes, lower thresholds, enhanced population spike and more synchronous firing were seen. In addition, a secondary population spike often occurred. There was less LFD under picrotoxin when comparisons were made with stimulus intensity held constant. Results with threshold held constant tended toward more depression under picrotoxin. Results are consistent with an increase in granule cell excitability through release of GABA mediated inhibition. (Supported by NIH grant NS22980-01A1 to PCR.)

334.5

RELATIONSHIP OF ELECTROGRAPHIC MEASURES TO BEHAVIOURAL SEIZURE DEVELOPMENT DURING AMYGDALOID KINDLING: A MULTIVARIATE ANALYSIS. G.C. Teskey, K.-P. Ossenkopp, and D.P. Cain. Dept. Psychology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C2.

During kindling, the most commonly employed electrographic measure used to assess seizure development has been duration of after-discharge (AD). However, several other electrographic measures can also be quantified.

Male Royal Victorian hooded rats were stimulated and AD recorded thru bilaterally implanted bipolar nichrome electrodes in the amygdala. Five different electrographic measures of AD were examined; i) duration, ii) number of spikes, iii) amplitude on stimulated side, iv) amplitude on contralateral side, and v) spike frequency. The five electrographic measures and Racine's behavioural classification of seizure stage were recorded on each session to give an indication of kindling progession. A multiple regression technique was used to assess the importance of each electrographic measure as a predictor of behavioural stage and the amount of variation which can be accounted for by each electrographic measure.

Results indicate that amplitude on the contralateral side correlates best with the behavioural seizure and can account for approximately 62% of the variation in seizure behaviour. Supported by NSERC.

334.6

ROLE OF AMYGDALOID COMPLEX IN SHORT AND LONG TERM EFFECTS OF REPEATED ELECTROCONVULSIVE SHOCK. V Leviel* C.Fayada* B.Guibert*, N.Faucon* G.Machek* and R Naquet. Lab de neurophysiologie, CNRS 91198 Gif sur Yvette, France

The short and long term effects of repeated electroconvulsive shocks were compared. To this purpose rats were submitted to a series of ten daily electroconvulsive shocks and sacrificed 24 Hr or 30 days after the last stimulation. Three different neurotransmi tory systems were investigated in discreet brain regions a)The enzyme activity of tyrosine hydroxylase (TH) and the messenger RNA (TH-mRNA) levels were measured. b)The activity of glutamic acid decarboxylase (GAD) was evaluated as well as the corresponding messenger RNA (GAD-mRNA) c)The tissue concentration of Met enkephalin and also the mRNA encoding for its precursor (PPE-mRNA) was determined

In the 24-hr group, nearly all of the parameters considered increased. TH-mRNA level increased two fold compared to the control value in the ventral tegmental area (VTA) as well as in the locus coeruleus (LC). The major effect on TH activity was in the central nucleus of amygdala (ACE) where increases of Met and PPE-mRNA were also detectable. Alterations in the enzyme or mRNA levels were not detected in the substantia nigra or in the striatum.

Analysing 30-day group, Met and PPE-mRNA remained higher than control in ACE, but, in contrast to the 24-hr group, TH activity and TH-mRNA quantities were returned to basal level in LC and were significantly decreased in VTA. Simultaneously, TH activity was also lowered in ACE

These results suggest that antidepressive therapy by repeated electroconvulsive shock could implicate an alteration in monoaminergic control of ACE from VTA and LC. The changes in TH-mRNA suggest the enzymatic induction as a possible mechanism of this alteration.

334.7

A POSSIBLE ANATOMICAL MARKER FOR ENDOGENOUS LTP. N. L Desmond and W. B Levy. Dept. Neurosurgery, Univ. Virginia Sch. of Med., Charlottesville, VA 22908.

LTP of the entorhinal cortical (EC)-dentate gyrus (DG) synaptic response correlates with morphological changes at these synapses. Previously we hypothesized that the concave spine synapses represent the population of potentiated synapses. Compared with the control synapses, a set of features is consistently associated with the potentiated synapses: a postsynaptic concavity at the synaptic interface, bigger spine heads, bigger PSD and membrane apposition surface areas, an increased number of front-line synaptic vesicles, wider spine stems, and a lower probability of a polyribosome at the base of the spine stem. In the absence of stimulation-induced LTP, this same set of synaptic features also distinguishes synapses in the DG so that EC-DG concave spine synapses normally are different from EC-DG noncon-cave spine synapses. For example, the mean membrane apposition surface area per concave spine synapse is 9.5 ± 0.7 um^2/100 um^3 while a simple spine synapse has on average 3.9 ± 0.2 um^2/100 um^3 membrane apposition surface area. Similarly, the mean number of front-line synaptic vesicles along the membrane apposition trace length is 9.3 ± 0.9 for the concave spine synapses and is 4.0 ± 0.4 for the simple spine synapses. Since the postsynaptic concavity correlates with the same set of features whether or not stimula-tion-induced LTP has occurred, we hypothesize that the concave spine syn-apses with their set of defining idiosyncratic features are markers for endogenous LTP. If true, the concavity of a spine synapse will identify associative synaptic potentiation, at least for those excitatory axospinous synapses using an acidic amino acid transmitter and possessing NMDA receptors, and can be used to identify LTP as a physiological correlate of learning. Supported by NIH NS15488 and NIMH RSDA MH00622 to WBL.

334.8

NOVEL MORPHOLOGICAL CHANGES IN HIPPOCAMPAL DENTATE GRANULE CELL PERIKARYA FOLLOWING RECURRENT LIMBIC SEIZURES. M.C.Bundman, R.M. Pico, J.Athanikar* and C.M.Gall. Departments of Anatomy and Neurobiology and Pharmacology, University of California, Irvine CA 92717

Recurrent limbic seizures stimulate increased enkephalin synthesis by the dentate gyrus granule cells and lead to changes in the synaptic vesicle populations within their mossy fiber terminal boutons which covary with alterations in enkephalin content. In the present study the influence of seizure activity on the ultrastructural morphology of the granule cell perikarya was examined.

Experimental rats received a unilateral electrolytic lesion in the dentate gyrus hilus; this treatment leads to intermittent limbic seizures which recur from 1.5 to 10 hrs postlesion. At 5 hrs postlesion, hilus lesion (HL) rats with behaviorally verified seizures and paired anesthetic-controls were sacrificed and the internal blade of dentate gyrus stratum granulosum contralateral to the lesion, was prepared for electron microscopy.

In HL rats, there was a striking increase in the number of somatic spines and points of association of the nuclear membrane (NM) with the granular endoplasmic reticulum (GER) . Granule cell somatic spines were rare in control rats (n=3; 5 spines/1,463µm plasma membrane from 62 cells) whereas in HL rats numerous well-elaborated somatic spines were easily identified (n=4; 36 spines/1,723µm plasma membrane from 73 cells). Moreover, a 3-fold increase in the number of NM/GER continuities was found in the HL rats (n=3; 170 continuities/1,074µm NM from 42 cells) as compared to controls (n=3; 57 continuities/1,038µm NM from 42 cells).

The present quantitative analyses demonstrate changes in the NM/GER association that are coincident with seizure-induced alterations in granule cell synthetic activities and indicate that hippocampal seizure activity stimulates the formation of new somatic spines on the dentate gyrus granule cells.
Supported by NSF grant BNS 8417098 and RCDA NS00915 to CMG

334.9

INCREASE IN THE RATIO OF "PERFORATED" TO "NONPERFORATED" SYNAPSES DURING HIPPOCAMPAL KINDLING IS SPECIFIC FOR THE SYNAPTIC FIELD OF STIMULATED AXONS. F. Morrell, Y. Geinisman and L. deToledo-Morrell. Depts. of Neurol. Sci. and Psychol., Rush Med. Coll. and Dept. of Cell Biol. & Anat., Northwestern Univ. Med. Sch., Chicago, IL 60612.

We recently demonstrated that an increase in the proportion of "perforated" to "nonperforated" synapses occurred in the terminal field of stimulated axons during hippocampal kindling (Morrell et al., *Epilepsia*, 1987, **28**: 617; Geinisman et al., *Proc. Natl. Acad. Sci. USA*, 1988, in press). The present study was designed to elucidate whether this structural synaptic modification was restricted to the terminal field of stimulated axons or whether neighboring but not directly stimulated regions showed the same modification. To answer this question, we performed a comparative analysis of the middle (MML) and inner (IML) molecular layer of the hippocampal dentate gyrus. The MML is a directly stimulated structure; the IML is not. The two are immediately adjacent synaptic fields likely to be equally susceptible to any generalized changes resulting from convulsions or anoxia. Rats were kindled via medial perforant path stimulation (1 msec pulses at 60 Hz for 2 sec, twice a day) and examined morphologically 4 weeks after reaching a criterion of 5 generalized seizures. Implanted but unstimulated rats served as controls. The number of synapses per neuron was assessed both in the IML and in the MML using the unbiased stereological disector technique. Axospinous synapses with continuous or discontinuous postsynaptic densities ("nonperforated" or "perforated" synaptic contacts) were differentially quantified. Results indicated that both "perforated" and "nonperforated" synapses were decreased in numbers (by 29 and 30%, respectively) in the IML of kindled rats compared with controls. Thus, there was no change in the ratio of one synaptic type to another. In the MML, on the other hand, a decrease in the number of "nonperforated" synapses was associated with a 46% increase in the ratio of "perforated" to "nonperforated" junctions in kindled animals. This finding supports the notion that the alteration in the ratio of the synaptic types during kindling is specific to the stimulated circuit. Such an alteration is not a consequence of nonspecific or generalized effects, but is rather a manifestation of neuronal plasticity tuned to the source of the synaptic drive.
Supported by Grant BNS-8607272 from NSF.

334.10

"PERFORATED" SYNAPSES ON DOUBLE-HEADED DENDRITIC SPINES: A STRUCTURAL MODIFICATION INDICATIVE OF SYNAPTIC PLASTICITY. Y. Geinisman, L. deToledo-Morrell and F. Morrell. Dept. of Cell Biol. & Anat., Northwestern Univ. Med. Sch. and Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, IL 60611.

Examination of axospinous synapses in serial sections obtained from the molecular layer of the rat dentate gyrus has revealed that some of them involve double-headed dendritic spines. Such a spine is attached to a parent dendrite by a single stalk. From the stalk emanates a neck which divides into two branches, each one terminating as a complex-shaped head. Each spine head is involved in a synaptic contact, and two synapses on a double-headed spine are invariably characterized by the following distinctive features: (1) they are formed by two separate axon terminals and (2) they belong to the type of "perforated" synapses distinguished by a discontinuous postsynaptic density. The convergence of two presynaptic axon terminals on a single spine may result in summation of their actions and extensive amplification of synaptic transmission. The latter effect may be further augmented by the existence of two "perforated" synapses on a single spine, since such synaptic contacts are believed to be more efficacious than "nonperforated" ones. The validity of the assumption regarding an amplification of synaptic action was tested in an experiment using kindling to induce a long-lasting enhancement of synaptic efficacy. Rats were kindled via medial perforant path stimulation and examined 4 weeks after reaching a criterion of 5 generalized seizures. Unstimulated but implanted rats served as controls. "Perforated" synapses on double-headed spines were quantified in the middle molecular layer of the hippocampal dentate gyrus with the aid of the unbiased stereological disector technique. Estimation of the number of these synapses per neuron showed that they constitute only about 2% of the total population of "perforated" axospinous synapses in control animals; in kindled rats, however, they were significantly increased in numbers (from 3.52 per neuron in controls to 6.05 in the kindled group). These results support the notion that "perforated" synapses on double-headed dendritic spines represent a structural modification related to enhanced synaptic efficacy.
Supported by Grant BNS-8607272 from NSF.

334.11

PARADOXICAL CHANGES IN SYNAPTIC TRANSMISSION IN THIAMINE DEPLETED RATS. N.W.Milgram, P.A. Nellis and G.O. Ivy. Div. of Life Sciences, Univ. of Toronto, Scarborough, Ontario, M1C1A4.

Rats were chronically prepared with stimulating electrodes in the perforant path and recording electrodes in the dentate gyrus using electrophysiological guidance to obtain evoked field potentials characteristic of this pathway. Following recovery, intensity response functions were taken and a curve fitting routine was used to quantify thresholds, rates of rise and asymptotic response levels. After establishing stability, the animals were placed on a thiamine deficient diet and treated daily with pyrithiamine (1mg/kg). The most striking effect was a dramatic potentiation in population spike amplitude (mean=75%) which only became apparent after about 10 days, coinciding with the appearance of acute neurological signs. Spike threshold, in contrast, increased gradually to an average level of 160%. In addition, the spike onset latency was either unaffected or increased. Histological analysis of the forebrain revealed a pattern of pathology encompassing the dorsal medial, ventral lateral and lateral dorsal thalamic nuclei. The concurrent increase in both amplitude and threshold is unprecedented, and may indicate that thiamine depletion affects both excitatory and inhibitory processes. Supported by NSERC.

ION CHANNELS: SODIUM CHANNELS II

335.1

DISTRIBUTION OF SODIUM CHANNELS ON DENDRITES OF IDENTIFIED VERTEBRATE NEURONS: IMMUNOCYTOCHEMISTRY AND ELECTROPHYSIOLOGY M. Ellisman, L. Maler, R.W. Turner, S.R. Levinson (UCSD, Univ. Ottawa, Univ. Colo. Boulder).

Sodium channels are responsible for initiating and propagating action potentials as well as participating in the setting of a neuron's threshold. It has been suggested that sodium channels are located primarily at nodes of Ranvier and the initial segment and that their density falls off rapidly over the proximal dendrites. We have used antibody directed against the sodium channel from electric eel electroplaque to examine the distribution of sodium channels on physiologically characterized, identifiable neurons in the brain of the related gymnotiform species, Apteronotus leptorhynchus. Our initial studies demonstrated that the sodium channel distribution is tightly controlled, being found over the somatic and dendritic surface of pacemaker cells but excluded from these regions in sensory neurons which are driven at the pacemaker frequency (Maler et al., '87). We now report on the distribution of sodium channels on pyramidal cells of the electrosensory lateral line lobe (ELL) first order sensory area for the electrosensory system. In vitro studies have shown that these neurons have, in addition to the action potential, two types of sodium dependent potentials: a slow persistent inward current and rapid small potential shifts resembling the D-spikes of hippocampal pyramids (Mathieson & Maler, in press). Immunocytochemistry (light microscopy, EM, and high voltage EM) on normal material and on physiologically characterized cells from the ELL slice filled with HRP, revealed that sodium channels were distributed in patches on both apical and basilar dendrites of the pyramidal cells, but were rarely found on the somatic surface. Purkinje cells located in the same tissue section had far fewer such patches on their dendritic surface. Localized application of TTX was employed to correlate the anatomic distribution of sodium channels and the electrical activity of these cells.

335.2

DIRECT IMMUNOGOLD LABELING OF Na^+ CHANNEL IMPs IN FREEZE-FRACTURE REPLICAS. J. E. Rash[1], T.J.A. Johnson[1*], J. E. Dinchuk[2*], D.S. Duch[3], and S.R. Levinson[4]. Depts. of Anatomy and Neurobiology[1] and Pathology[2], Col. State U., Ft. Collins, CO 80523, Dept. of Anesthesiology3, Cornell Univ. Med. Sch., NY, 10021, and Dept. of Physiology[4], U. Col. Health Sci. Center, Denver, CO 80262.

Reconstituted membrane vesicles containing purified Na^+ channels from E. electricus as their only proteins were freeze-fractured and replicated. Replicated vesicles were thawed in "labeling blocking buffer," labeled with rabbit anti-Na^+ channel polyclonal Ab, counter-labeled with affinity-purified goat anti-rabbit Ab on 10 nm gold, and rinsed carefully. In these "replica-label-whole mounts," <10% of the area was occupied by vesicles and >90% by extravesicular matrix, but >90% of gold granules were associated with vesicles. Of this "vesicle specific" labeling, ca. 25% were within 20 nm of IMPs, ca. 25% were attached to unreplicated portions of vesicles (where Na^+ channel proteins were equally likely), ca. 25% were associated with deeper (unreplicated) layers of multilamellar vesicles, and ca. 15% were not associated with IMPs. Occasionally, distinctive co-patterning of IMPs and gold granules was discerned. To estimate "labeling efficiency," we presumed that Na^+ channel proteins are oriented at random in the membrane bilayer, thereby eliminating 50% from potential labeling. Of the remaining sites, approximately 30% were labeled. Based on relative ratio of apparent specific to non-specific labeling, proximity of labels to IMPs, and "sidedness" as revealed in stereoscopic images, we conclude that Na^+ channel IMPs in a simple model system were identified by direct immunogold labeling.

Supported by grants from NIH (NS-15991 to JER and NS-15879 to SRL) and by a contract from DoD (DAMD-17-84-C-4010).

335.3

IMMUNOCYTOCHEMICAL LOCALIZATION OF SODIUM CHANNEL SUBTYPES R_I AND R_{II}. R.E. Westenbroek* and W.A. Catterall . Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195.

The voltage sensitive sodium channel is a transmembrane protein responsible for the rising phase of the action potential in electrically excitable tissue. Affinity-purified anti-peptide antibodies distinguish between the α subunits of sodium channel subtypes R_I and R_{II} in rat brain, and immunoprecipitation experiments have shown that R_I and R_{II} are expressed primarily in the central nervous system with R_{II} predominant in brain and R_I predominant in spinal cord (Gordon et al, *PNAS* 84:8682-8686). These antibodies have been used in combination with the indirect peroxidase-anti-peroxidase technique to investigate the distribution of R_I and R_{II} in the brain and spinal cord of adult rats. Light microscopic studies have revealed that the overall immunoreactivity for R_{II} decreases from rostral to caudal with intense staining in several brain regions and diminished staining in the spinal cord. Throughout the extent of the substania nigra there is dense labeling of fibers. In the CA1, CA2 and CA3 regions of the hippocampus, axons, but not the somata, of pyramidal neurons are immunoreactive for R_{II}. Dense immunoreactivity is present in the molecular layer of the cerebellum containing Purkinje cell axons. However, the cell bodies of Purkinje cells, interneurons and granule cells are not labeled. In addition, punctate staining of neurons and their processes is observed in the supraoptic and paraventricular nuclei. Throughout the extent of the spinal cord, R_{II} positive fibers are present mainly in the dorsal laminae 2 and 3 with relatively little immunoreactivity observed in the ventral horn.

Immunoreactivity for R_I is most apparent in the spinal cord. There is a dense band of R_I positive fibers and/or terminals located mainly in lamina 2 and/or 3, with general immunoreactivity throughout the remainder of the dorsal and ventral horns. The somata of neurons located in the ventral horn are also labeled by R_I antibodies. Overall, our results illustrate a complex distribution of the R_I and R_{II} subtypes among individual cell groups and fiber tracts in the central nervous system.

335.4

Na CHANNELS IN FETAL DIFFERENTIATING NEURONS.
M. R. WOOD, G. STRICHARTZ*, L. ELMER**, K. ANGELIDES** and K.H. PFENNINGER, UNIV. COLORADO HEALTH SCIENCES CENTER, DENVER, CO. 80262. *HARVARD MEDICAL SCHOOL, BOSTON, MA. 02115. **BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX. 77030.

Previous studies on STX/TTX binding to CNS growth cones (GCPs) from E18 fetal rats have demonstrated both high- and low-affinity Na channels (K_D 0.32, 4.0 nM). In comparable synaptosomal preparations, only a high-affinity binding site (K_D 0.76 nM) was demonstrable, but it was much more abundant than all binding sites in GCPs (Wood et al., 1987, J. Cell Biol. 105, 143a).
We now report data from competition experiments indicating that there are no TTX-resistant STX binding sites present in GCPs. Western blots of synaptosomes and GCPs were treated with polyclonal Na channel antibodies and ^{125}I-second antibody. This revealed an equally high level of immunoreactivity in GCPs compared to synaptosomes with the same protein loading. Antibodies to specific synthetic peptides of rat brain Na channels recognize polypeptides of different M_r in synaptosomes versus GCPs. This provides further evidence for a different type of Na channel in growth cone membranes. These findings are relevant to the maturation of neuronal plasmalemma and the development of its functional domains. (Supported by NIH NS-24676 grant to K.H.P.)

335.5

ANTIBODIES AGAINST A CONSERVED INTRACELLULAR SEGMENT SLOW SODIUM CHANNEL INACTIVATION. P.M. Vassilev*, T. Scheuer* and W.A. Catterall. (Spon: M. Ahlijanian) Dept. of Pharmacology, SJ-30, University of Washington, School of Medicine, Seattle, WA 98195.

The primary amino acid sequence of the α-subunits of Na channels in some excitable membranes has been defined, but the relationships between molecular structure and mechanisms of channel gating remain unclarified. The effects of four antibodies, directed against different predicted intracellular segments of the α-subunits, have been tested on whole cell sodium currents in rat skeletal muscle cells. An affinity-purified antibody (anti-SP19) directed against a highly conserved amino acid sequence in the intracellular segment between domains III and IV (residues 1540 to 1557 of R_{II}) induced a substantial slowing of the inactivation kinetics which was blocked by the corresponding peptide. At a holding potential of -110 mV the intracellular application of the antibody increased the time constant of the Na current decay from 2.28 to 4.37 ms during a test pulse to -20 mV. A similar effect was observed at a holding potential of -70 mV, but its onset following seal formation occurred at a three-fold slower rate than at -110 mV. A dependence upon the test pulse potential was also observed. Slowing of inactivation was small for pulses to -50 mV, but was more pronounced at more positive test potentials up to 90 mV. Antibodies directed against distinct amino acid sequences in other intracellular segments of the R_{II} α-subunit did not significantly affect Na channel inactivation. These results identify a highly conserved intracellular segment between domains III and IV of the α-subunit which may be involved in the channel inactivation mechanism.

335.6

MOLECULAR MAPPING AND CLONING OF THE *DROSOPHILA TIP-E* LOCUS -- A MUTATION AFFECTING VOLTAGE-SENSITIVE SODIUM CHANNELS. D. W. Gil*, D. P. Kasbekar*, P. Deak* and L. M. Hall. Dept. of Genetics, Albert Einstein College of Medicine, Bronx, NY 10461.

The *tip-E* mutation causes temperature-induced paralysis, reduces the number of saxitoxin binding sites in membrane extracts and decreases the sodium current in patch clamped embryonic neurons (O'Dowd, D. K. and Aldrich, R. W., J. Neurosci., in press). In order to understand how the *tip-E* gene product affects sodium channel properties, we are cloning the gene by chromosome walking.

We have generated chromosome rearrangements that uncover the *tip-E* phenotype and used them to localize the gene to a region (64A4-B12) on the left arm of the third chromosome. In addition to *tip-E*, this region contains several other neuronally expressed genes, including a *Drosophila ras* oncogene and an acetylcholine receptor subunit homolog. We have mapped these genes with respect to the chromosome rearrangement breakpoints by hybridizing them to genomic Southern blots and to salivary gland squashes. Using these clones as starting points for a walk through the *tip-E* region, we are identifying clones that cross the breakpoint of a translocation that results in a *tip-E* phenotype. We will then use transposon mediated transformation to rescue *tip-E* embryos with clones that span this translocation break.

335.7

CLONING AND CHATACTERIZATION OF A NOVEL VOLTAGE-GATED SODIUM CHANNEL. V. Auld+*, A. Goldin'*, D. Krafte'*, J. Marshall+*, J. Dunn+*, W. Catterall"*, H. Lester'* N. Davidson'*, and R. Dunn+*. (SPON: K. Johnston) +Dept. Medical Genetics, University of Toronto, Toronto, M5S 1A8, 'Church Chemical Laboratories, CalTech, Pasadena, CA 91125 "Dept. Pharmacology, University of Washington, Seattle, WA 98195.

We have cloned and constructed a full length cDNA encoding the rat brain Na channel ∝ subunit. The encoded ∝ subunit differs from the Rat2 ∝ subunit of Noda et al (Nature, 320:188, 1986) at six amino acid positions. RNA was transcribed in vitro from this cDNA and injected into Xenopus oocytes to produce functional sodium channels. Although the primary sequences of the two ∝ subunits were almost identical, the current-voltage relationship for channels produced from our cDNA was shifted 20-25 mV in the depolarizing direction compared to that reported for the Rat2 subunit (Stuhmer et al, Eur. Biophys. J., 14:131, 1987). It is likely that one or a combination of the six varient amino acids is responsible for this shift in the current-voltage relationship. Each of the amino acids has been altered by site directed mutagenesis to the corresponding residue found in the Rat2 ∝ subunit. Voltage clamp analysis of the channels produced from these mutants provides evidence for the location of structural domains in the sodium channel subunit which are important for the voltage dependent channel opening.

335.8

SUBTYPE-SELECTIVE BLOCK OF SODIUM CHANNELS BY ZINC STUDIED AT THE SINGLE CHANNEL LEVEL IN PLANAR BILAYERS. A. Ravindran* and E. G. Moczydlowski. Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The divalent cation, Zn, has been implicated as a subtype-specific inhibitory modulator of glutamate-activated channels in mammalian brain. In what may be an analogous situation, certain Na-channel subtypes that exhibit low affinity for tetrodotoxin (TTX) have been found to be more sensitive to Zn than TTX-sensitive subtypes. We compared the effect of Zn and other divalent cations on batrachotoxin-activated Na-channels from rat skeletal muscle (TTX-sensitive) and canine heart (TTX-insensitive) after incorporation into planar lipid bilayers. External Mg, Ca, Sr, Ba, Mn, Co and Ni induced a fast voltage-dependent block (decreased unit conductance) in both types of channels. The following values were obtained for apparent blocking K_D's (in mM) at 0 mV and symmetrical 0.2 M NaCl, pH 7.4: Mg (48), Ca (40), Sr (72), Ba (88), Mn (18), Co (13), Ni (15) for muscle and Mg (51), Ca (41), Sr (75), Ba (76), Mn (20), Co (15), Ni (13) for heart. In contrast to the similar affinity of these cations for the two subtypes, Zn blocked the muscle subtype with a low affinity (K_D =11 mM) and the heart subtype with a 120-fold higher affinity (K_D =0.09 mM) at 0 mV. In contrast to a fast block by Zn for the muscle channel, Zn induced brief closing events in the heart channel with a mean duration of about 20 ms from -60 to +60 mV. The frequency of Zn-induced blocking events was both concentration and voltage dependent, indicating a voltage-dependent association rate for Zn. (NIH AR38796)

335.9

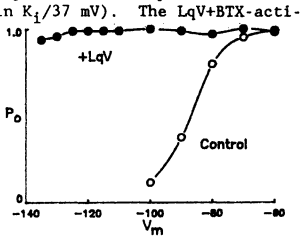
DIFFERENTIAL SENSITIVITIES TO Na+-CURRENT BLOCKADE BY TTX AND COCAINE IN N1E-115 AND RAT DORSAL ROOT GANGLION (DRG) CELLS. **J.M. Frey, K.C. Chinn*, C.P. Bianchi and T. Narahashi. Depts. of Pharmacology and Medicine/ Div. Toxicology, Northwestern Univ. Med. Sc., Chicago, IL 60611 and Thomas Jefferson Med. Col., Philadelphia, PA 19107.**

The convulsant properties of cocaine are well known and may involve the disinhibition of neuronal pathways within brain. We have examined the effects of cocaine HCl (COC) and TTX on Na+ currents by the whole cell patch clamp technique in two cell populations, the mouse neuroblastoma cell line N1E-115 (NB) grown in cell culture and DRG neurons acutely dissociated in papain/collagenase-disp./cysteine. Peak Na^+ currents (I_{Na}) were compared in experiments conducted at room temperature with Co++ and Cs++ to eliminate Ca++ and K+ currents. Peak inward I_{Na} averaged 4.5±0.8 and 3.7±0.8(SEM)nA in NB and DRG, resp. DRG neurons were less sensitive to TTX, requiring at least 5μM and up to 10μM to eliminate I_{Na}. In NB, TTX produced complete block at 1μM. COC block was time-dependent and irreversible, shifting Na+ IV curves up and to the right with maximal effect at 20-30min in both cell populations. In NB, COC (10^{-4}M) blocked I_{Na} by 39.3±3.2% at 20 min. In DRG, the same concentration produced 89.0±1.7% block. NB cells incubated in COC at least 1 hr and then patched, exhibited I_{Na} >2.5nA, whereas I_{Na} in 70% of DRG were blocked to <1nA. I_{Na} was slower in DRG than in NB. Certain cell populations may be at risk to COC even though they may exhibit low sensitivity to classical Na+ channel blockers.

335.10

GATING OF BATRACHOTOXIN-ACTIVATED Na CHANNELS IS ALTERED BY SCORPION VENOM IN PLANAR LIPID BILAYERS. M. E. O'Leary* and B. K. Krueger, Dept. of Physiology, Univ. Maryland Sch. of Med. Baltimore, MD 21201.

The effects of *Leiurus quinquestriatus* scorpion venom (LqV) on rat brain Na channels were studied in planar bilayers. In the presence of batrachotoxin (BTX), Na channels do not inactivate, however, BTX-activated Na channels still display voltage-dependent activation with the midpoint of the open probability (P_o) vs V_m relation about -90 mV. In the presence of BTX, LqV altered channel gating so that P_o remained > 0.95 at hyperpolarized potentials to -135 mV, the most negative potential tested. The single channel conductance (25 pS in symmetrical 250 mM NaCl) was not affected by LqV, moreover, LqV+BTX-activated channels displayed normal voltage-dependent block by nanomolar external STX (e-fold change in K_i/37 mV). The LqV+BTX-activated channels were also blocked by external Ca^{2+} in a voltage-dependent manner. The results suggest that even with inactivation removed by BTX, LqV alters Na channel gating without affecting site 1 toxin binding or Na^+ permeation. Supported by NIH & USAMRDC.



335.11

PRESENCE AND SIGNIFICANCE OF TETRODOTOXIN SENSITIVE SODIUM CHANNELS IN CAROTID BODY CHEMORECEPTOR CELLS. A. Rocher*, A. Obeso*, C. Gonzalez and B. Herreros* (SPON: M. Rodrigo Angulo). Depto. Bioq. Biol. Mol. y Fisiol. Fac. Med. Universidad de Valladolid. 47005-Valladolid (Spain).

There are clues that depolarization of chemoreceptor cells is an early event in their secretory response when stimulated by low pO_2. We explored the presence in type I cells of tetrodotoxin (TTX)-sensitive Na^+ channels as devices to generate the suspected depolarization.

Rabbit carotid bodies (c.b.) were loaded with ^{3}H-dopamine (^{3}H-DA) by incubation with 20 uM ^{3}H-tyrosine. ^{3}H-DA loaded c.b. were superfused with saline solutions at different pO_2 tensions or with veratridine either with or without TTX. We found: 1) Veratridine evokes ^{3}H-DA release in a dosis-related fashion. 2) Veratridine evoked release was dependent on Na^+_o and Ca^{++}_o. 3) Veratri- (50 uM) evoked release was blocked by TTX (1 uM). 4) Low pO_2 (0-40 torr) evoked release was partially inhibited by TTX (1 uM), being greater the inhibition at lower pO_2. These findings indicate that chemoreceptor cells posseses Na^+ channels and their involvement in the physiological stimulus-secretion reaction.

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335.12

CARDIOVASCULAR EFFECTS OF 1-METHYL-4-(1-NAPHTHYLVINYL) PIPERIDINE HYDROCHLORIDE T.R. Henderson, E. M. DeLorme, K. Takahashi, A. P. Gray and K. L. Dretchen. Dept. of Pharmacology, Georgetown Univ. Sch. of Med. and Dent., Washington, D.C. 20007 and the Dynamac Coproration, Rockville, MD 20852.

1-Methyl-4-(1-naphthylvinyl) piperidine (B-120), in a dose range of 1 to 10 mg/ kg, produced up to a 70% decrease in systolic, diastolic and mean blood pressure without a corresponding change in heart rate in cats. The hypotensive effects were still observed in the presence of hexamethonium and atropine. B-120 did not alter the cardiovascular response to acetylcholine or vagal stimulation. Also, it did not alter the response of the nicti tating membrane to either pre- or post-ganglionic stimulation. In a rat cortical synaptosome preparation, B-120 (100 um), diminished both potassium-stimulated and basal calcium flux by 75%. In this regard, B-120 was equipotent to verapamil. B-120 has been previously reported to reduce organophosphate toxicity (J. Med. Chem. 31:807, 1988) in mice and guinea pigs. It seems likely that the organophosphate protection seen with B-120, as well as its hypotensive effects, may be related to its capacity to block calcium channels.

[This work was supported by contract No. DAMD17-82-C-2167 from the Army Medical Research and Development Command]

335.13

INDUCTION OF STX-SENSITIVE SODIUM CHANNELS IN CULTURED ASTROCYTES. P.J. Yarowsky, D.S. Brougher* and B.K. Krueger. Depts. of Pharmacology & Experimental Therapeutics and Physiology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201

Tracer flux and STX binding studies reveal a progressive change in the STX affinity of cultured neonatal rat astrocyte Na channels. Low STX affinity Na channels (K_d > 40 nM) were present from the first through the fourth week in culture. High STX affinity Na channels (K_d = 0.4 nM) were present at very low levels during the first week, but increased rapidly over the next 5 days reaching a maximum (2.2 pmol/mg protein) by the end of the second week. This spontaneous change in STX affinity was coincident with changes in morphology, from polygonal cells with few processes to stellate cells with numerous processes. Replacing standard medium (MEM + 10% FCS) with serum-free, chemically defined medium at day 7 induced morphological differentiation within 15 hr in all cells. This change in medium also rapidly increased the proportion of high STX affinity channels to 62% at a time when they normally constituted only 5%. Although serum-free medium promotes the appearance of oligodendrocytes, we found no saturable STX binding to purified cultures of oligodendrocytes. Since low STX affinity channels were still present in these chemically defined cultures, both high and low STX affinity Na channels probably exist in mature stellate astrocytes. (Support: NIH and NSF)

335.14

INTENSITY-DEPENDENT SHIFT IN THE SITE OF ORIGIN OF ORTHODROMIC ACTION POTENTIAL DISCHARGE IN CA1 HIPPOCAMPAL PYRAMIDAL NEURONS. R.W. Turner*, D.E.R. Meyers and J.L. Barker. Lab. of Neurophysiology Nat. Inst. Health, Bethesda, MD 20892 (SPON: K.C. Marshall)

Synaptic depolarization of CA1 hippocampal pyramidal cells (HPC) is thought to evoke Na^+-dependent spike discharge at both the axon hillock and one or more dendritic sites containing a high density of Na^+ channels. The present study examined the site of origin of stratum radiatum (SR)-evoked spike discharge in the rat hippocampal slice preparation. HPC somatic and dendritic spike discharge was examined by testing the sensitivity of intra- and extracellular potentials recorded in the cell body layer (soma/axon hillock) and proximal apical dendrites to local application of TTX (40 μM).

At moderate to high stimulus intensities SR stimulation evoked a fast negative population discharge in proximal dendrites with shorter peak latency than the cell body population spike. TTX ejection to the somatic region reduced the cell body population spike with little change in the proximal dendritic negativity. In contrast TTX restricted to proximal dendrites reduced both the dendritic and cell body population discharge. At threshold, SR-evoked proximal intradendritic spikes were blocked in an all-or-none manner by TTX ejection to the cell body layer. At higher intensities the same ejection abolished the cell body population spike but reduced the intradendritic spike by <20%. Blockade of the intradendritic spike occurred subsequent to diffusion of TTX to the proximal dendrites.

The results suggest that at threshold SR-evoked spike discharge originates in or near the cell body layer. Higher intensities of activation shift the site for spike initiation from the cell body layer into the proximal apical dendritic region.

335.15

[^{14}C]GUANIDINIUM ION INFLUX INTO MOUSE BRAIN SYNAPTOSOMES AS A MODEL FOR $^{22}Na^+$ ION INFLUX M.E.A. Reith. Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York, NY 10035.

Voltage-dependent sodium channels are permeable to a number of small organic cations including guanidinium ions. Indeed, a recent report (Eur. J. Pharmacol. 124(1986)291) indicated similarities between [^{14}C]guanidinium ion and $^{22}Na^+$ ion influx into crude synaptosomes from rat brain. In the present study [^{14}C]guanidinium ion influx into purified synaptosomes from mouse cerebral cortex was characterized in more detail in Na^+ free medium (Mol. Pharmacol. 19(1981) 78). Veratridine-activated [^{14}C]guanidinium influx, corrected for flux unrelated to sodium channels measured in the presence of 1 μM tetrodotoxin, was linear with time up to 30 sec and with guanidinium ion concentration up to 6 mM. Activation of [^{14}C]guanidinium ion (3 mM) influx (30 sec) was half-maximal at 3 μM of veratridine. Scorpion venom (20 μg/ml) shifted the activation curve to the left (half-maximal stimulation at 0.1 μM). Local anesthetic drugs inhibited guanidinium ion influx with potencies depending upon the concentration of veratridine used for activation, and inhibition was fully overcome by increasing concentrations of veratridine, suggesting a competitive interaction in contrast to the findings reported for Na^+ ion influx. Despite this difference, [^{14}C]guanidinium ion influx appears to be a valid model for $^{22}Na^+$ ion influx.

I thank the NIDA for support (grant DA 03025) and Irma Vara-Reith for technical assistance.

335.16

LOCAL ANESTHETICS: INHIBITORY EFFECTS ON BATRACHOTOXIN-ELICITED SODIUM FLUX AND PHOSPHOINOSITIDE BREAKDOWN IN GUINEA PIG CEREBRAL CORTICAL SYNAPTONEUROSOMES. Y. Nishizawa*, F. Gusovsky and J.W. Daly. Lab. of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD 20892

Local anesthetics inhibit the sodium influx and the inositol phosphate accumulation elicited by the sodium-channel activator batrachotoxin in guinea pig cerebral cortical synaptoneurosomes. Inhibitory effects of local anesthetics on sodium influx correlate closely with inhibitory effects on binding of a tritiated batrachotoxin analog to sodium channels in synaptoneurosomes. Inhibitory effects of local anesthetics on sodium influx also correlated with inhibitory effects on inositol phosphate accumulation. Euprocin, bupivacaine, lidocaine and certain analogs were nearly equipotent with respect to inhibition of sodium influx and inositol phosphate accumulation. Local anesthetics also inhibited inositol phosphate accumulation that was induced by carbamylcholine through both a tetrodotoxin-sensitive and a tetrodotoxin-insensitive pathway. Certain local anesthetics, such as dibucaine, inhibited the tetrodotoxin-sensitive pathway with higher potency than for the tetrodotoxin-insensitive pathway, while others, such as quinacrine inhibited tetrodotoxin-sensitive and insensitive pathways with equal potency. The data support an involvement of sodium channels in regulation of phosphoinositide breakdown in synaptoneurosomes, but indicate that effects of certain local anesthetics on muscarinic receptors or on the phospholipase C system may complicate interpretations.

335.17

MODULATION BY VERATRIDINE, BATRACHOTOXIN AND MONENSIN OF PHOSPHOINOSITIDE HYDROLYSIS IN NEUROHYBRID NCB-20 CELLS. D.-M. Chuang, Lab. Preclin. Pharmacol. National Inst. of Mental Health, St. Elizabeths Hospital, Washington, D.C. 20032.

Two sodium channel activators, veratridine and batrachotoxin (BTX), and a sodium ionophore, monensin activated phosphoinositide hydrolysis measured by ^{3}H-inositol monophosphate (IP_1) accumulation in NCB-20 cells. The % stimulation by these agents was unaffected by depleting extracellular NaCl suggesting that sodium influx is not necessary for this activation. In sodium-free medium, the stimulation by BTX and monensin was apparent in the presence of 60 or 10 mM lithium. Tetradotoxin blocked effects of BTX and veratridine but not that of monensin. The effects of these agents were dependent on extracellular calcium. The BTX effect was inhibited by micromolar nimodipine, while the monensin effect was unchanged. Ouabain did not alter phosphoinositide turnover. BTX and monensin also induced greater than additive effect on carbachol-induced ^{3}H-IP_1 accumulation. In cells with a high passage number, veratridine markedly inhibited the response of carbachol with 8-10 fold increase in the EC_{50}. This inhibition is mainly due to a defect in the receptor-effector coupling rather than in the binding of agonists to cell surface muscarinic receptors.

335.18

PHOSPHOINOSITIDE HYDROLYSIS INDUCED BY SODIUM CHANNEL ACTIVATORS IN MOUSE BRAIN: ROLE OF Na^+,Ca^{2+}, AND MEMBRANE POTENTIAL. M. Benuck*, M.E.A. Reith and A. Lajtha.(SPON: L.J. Cote) Ctr. Neurochem.,NS Kline Inst.,Ward's Isl.,NY 10035.

Veratridine (VERA), batrachotoxin (BTX) and other Na^+ channel activators are known to increase phosphatidylinositol (PI) hydrolysis in brain. The present work with mouse cerebrocortical slices addresses as possible mechanisms 1) the entry of Na^+, 2) depolarization, and 3) Ca^{2+} influx through voltage-dependent Ca^{2+} channels. Release of neurotransmitters is not supported by previous results.

Depolarization by 30 mM KCl enhanced PI hydrolysis. This response was not blocked by tetrodotoxin (TTX) and not stimulated by Ca^{2+} channel agonists (10 μM Bay K 8644) or prevented by Ca^{2+} antagonists (10 μM nimodipine or PN 200-110), indicating the lack of involvement of Na^+ and Ca^{2+} channels in the KCl effect. The PI response to VERA (10 μM) was blocked by TTX but not affected by the Ca^{2+} agents. The PI response to KCl was not additive with that to VERA or BTX, suggesting a common mechanism. This could be the accumulation of intracellular Na^+ ions, in the case of KCl by means other than Na^+ channels. Consonant with this possibility is the increase in PI hydrolysis found with 3 μM monensin promoting Na^+ influx without inducing depolarization, and the lack of the PI response to 30 mM KCl, 10 μM VERA, and 10 μg/ml of ScV in sodium-free media. In the latter case, a positive response to 2 mM carbachol demonstrated the functional activity of the PI system. These results support a direct role for Na^+ in regulating PI turnover. (DA 03025)

335.19

INTERACTION OF AN INSECTICIDAL ISOBUTYLAMIDE WITH VOLTAGE-SENSITIVE SODIUM CHANNELS. J. A. Ottea*, G. T. Payne*, J. R. Bloomquist, and D. M. Soderlund. Dept. of Entomology, NYS Agric. Expt. Station, Cornell University, Geneva, NY 14456.

BTG 502 [(2*E*,4*E*)-*N*-(1,2-dimethyl)-propyl-6-(5-bromonaphth-2-yl)-hexa-2,4-dienamide] is a synthetic analog of insecticidal amides isolated from *Piper* species (Elliott, M., et al. *Agric. Biol. Chem.* 50: 1347, 1986). BTG 502 at 10 μM produced approximately 75% suppression of evoked compound action potentials in excised mouse sciatic nerves. This compound also caused a slight stimulation of $^{22}Na^+$ uptake into mouse brain synaptoneurosomes, which was increased by saturating concentrations of *Leiurus quinquestriatus* venom. In the presence of *Leiurus* venom, half-maximal stimulation was achieved at 3 μM and maximal stimulation (2.3-fold greater than nonspecific uptake) at 50 μM. In the absence of *Leiurus* venom, BTG 502 inhibited veratridine (100 μM)-dependent $^{22}Na^+$ uptake. BTG 502 also inhibited the specific binding of [^{3}H]batrachotoxinin A-20-α-benzoate (BTX-B) to sodium channels in these preparations, producing half-maximal inhibition at 2 μM and maximal inhibition at 30 μM. The slope of the Hill plot for the displacement of [^{3}H]BTX-B by BTG 502 was substantially less than 1, suggesting that the effects of BTG 502 involve a negative allosteric interaction with the activator recognition site of the sodium channel. The effects of BTG 502 on $^{22}Na^+$ uptake and [^{3}H]BTX-B binding differ from those of other lipophilic insecticides (e.g., DDT and pyrethroids) and suggest the existence of a second insecticide binding domain on the sodium channel.

335.20

VOLTAGE-DEPENDENT POTASSIUM, SODIUM AND CALCIUM CURRENTS IN SMALL-CELL LUNG CANCER CELLS. J.J. Pancrazio*, M.P. Viglione*, I. Tabbara* and Yong I. Kim. (SPON: C. Desjardins) Depts. of Neurology, Biomed. Eng. & Int. Med., U.Va. Sch. of Med., Charlottesville, VA 22908.

Small-cell carcinoma (SCC) of the lung is a highly malignant tumor associated with a variety of paraneoplastic syndromes. Little is known about the types of ion channels present in these neuroendocrine-like cells.

Using patch clamp technique, we measured whole-cell currents from NCI-H128 and H69 SCC cell lines, held at -80 mV and depolarized to -60 to +120 mV (range of cell membrane capacitance: 4.0-24.8 pF). The outward K^+ current (I_K) was most prominent and found in every cell tested. I_K, measured in the presence of TTX at +80 mV, ranged from 0.9 to 4.3 nA. Externally applied TEA (30 mM) and 4-AP (2 mM) reduced I_K by about 90%. The inward sodium current (I_{Na}) was recorded after blocking I_K with internally applied CsCl and TEA. The rapidly inactivating I_{Na}, present in 25% of the cells studied, had a peak amplitude of about 70 pA (at 0 mV). I_{Na} was sensitive to TTX and reversed its polarity at 40-50 mV. Inward Ca^{2+} current (I_{Ca}), measured with 10 mM Ca^{2+} buffer after blocking I_{Na} and I_K, was readily detectable in H128 but less obvious in H69. I_{Ca} peaked at 7-10 msec with an amplitude of 10-120 pA, partially inactivating over the 40 msec depolarization (Supported by NIH grant NS18607 and an MDA research grant).

INVERTEBRATE LEARNING AND BEHAVIOR II

336.1

INTRACELLULAR SIGNALS PRODUCING HABITUATION IN STENTOR. David C. Wood. Department of Behavorial Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

The contractile protozoan, Stentor, habituates during repeated mechanical stimulation due to a progressive decrement in receptor potential amplitude. Repetitive elicitation of action potentials and contractions is required to produce this decrement; repetitive elicitation of receptor potentials alone produces no decrement. After responding to the first 3 to 10 stimuli, an occasional animal ceased contracting despite continued elicitation of receptor and action potentials. In these cases the partially decremented receptor potential recovered to prestimulation levels. Therefore, the contractile mechanism generates an intracellular signal which modifies the mechanoreceptor channels and produces habituation.

Increased intracellular Ca^{++} triggers contractions, but this Ca^{++} is not the signal for habituation since receptor potentials decrement normally when recorded with 50 mM EGTA-filled electrodes. EGTA did prevent Ca^{++}-dependent inactivation of the voltage-dependent Ca^{++} channels.

Twenty different drugs, toxins and ions known to modify the efficacy of second messenger systems were screened for their ability to alter habituation in Stentor. Only phosphodiesterase inhibitors and dibutryl cAMP increased the animal's rate of habituation without affecting their sensitivity to photic, electrical or temporally spaced mechanical stimuli. Therefore, cAMP may serve as a second messenger in the production of habituation in Stentor.

336.2

FOOD MODULATES SPIKE ACTIVITY IN FEEDING CELLS IN THE LEECH. T. Karrer and C.L. Sahley, Psychology and Biology, Yale University, New Haven, CT 06520.

Last year we reported that a carnivorous leech, *Haemopis marmorata*, could learn to alter its food preferences as a result of associative conditioning. As a first step toward the elucidation of the neurobiological events underlying this learning, we have investigated the effects of food on the LL cell, a cerebral cell which in *Hirudo medicinalis* has been shown to play an integral part in the mediation of feeding behavior (Lent & Dickinson, 1984).

Our preparation consisted of the sub- and supra-esophageal ganglia, the two most anteriorly-projecting pairs of cephalic nerves, and a piece of lip containing putative chemosensory structures (Elliott, 1986). The lip was sealed in a separate chamber. Also attached was the dorsal portion of the nerve ring which innervates the pharynx, and the previously unreported pharyngeal ganglia, which lie at the bifurcation point of the pharyngeal ring.

The LL is usually spontaneously active, producing single spikes at a mean frequency of 0.6 Hz. Food applied to the lip results, at a mean latency of 20 min, in the spikes changing to doublets at an approximate frequency of 1 Hz. This change generally persists for about 40 min.

To determine whether this change is presynaptic, or whether it is due to an intrinsic change in LL, we bathed the CNS in high Mg++ throughout the food application. The Mg++ abolished the spontaneous activity, and evoked spikes remained single. However, after washing out the Mg++, the spikes changed to doublets. This suggests that the change is presynaptic to the LL cells.

336.3

ASSOCIATIVE LEARNING IN THE LEECH: BEHAVIORAL AND CELLULAR EFFECTS OF PREDICTABILITY. Christie L. Sahley, Dept. of Biology, Yale Univ, New Haven, CT 06511.

The pairing and the predictive relationships between stimuli have been shown to be critical variables in associative learning for both vertebrate and invertebrate species. We previously addressed the pairing dependency in the intact leech. In this study we used a semi-intact preparation to address both of these variables and their cellular correlates.

Our behavioral procedures were similar to those originally used to study contingency in vertebrates (Rescorla, 1967). We compared the learning performance of leeches in four groups. Leeches in Group (Grp) 1 received 30 CS-US pairings (touch-shock). This procedure results in a significantly enhanced response to the CS. Leeches in Grp 2 experience the same pairings and they received 12 presentations of the US alone. Grps 1 and 2 were matched for US frequency. The additional US's degraded the predictive CS-US relationship. Leeches in this group show significantly less learning. Since leeches in Grp 2 received more US's than leeches in the paired grp (1), a third grp was run which received 42 paired CS-US presentations, thus controlling the total number of US presentations. Leeches in Grp 3 showed learning comparable to the learning observed in Grp 1. Finally, leeches in Grp 4 received 30 random presentations of the CS and US. Leeches in this group showed a decrement in behavior.

Cellular experiments demonstrate that the Retzius cell (R) may be implicated in this phenomenon. First, the R cell fires in response to the US and second, the firing decrements to repeated US alone presentations. No decrement has been observed during pairings. The decrement may be mediated through autoreceptors on the Retzius cell.

336.4

HABITUATION OF THE TRITONIA ESCAPE SWIM INVOLVES MULTIPLE BEHAVIORAL MODIFICATIONS. G. Brown*, W.N. Frost and P.A. Getting (SPON: M.J. O'Donovan). Dept. Physiol. & Biophys., Univ. of Iowa, Iowa City, IA. 52242.

Previous work has shown that habituation of the *Tritonia* escape swim involves a decrease in the number of cycles per swim. We now report that habituation is associated with changes in three additional aspects of the swim. During 10 trials (2 min intertrial interval) using noxious epithelial stimuli to trigger swimming, the duration of the first cycle increased from an average of 5.9 seconds (1st trial) to 7.7 (10th trial) ($N=7$, $p<.001$). In addition, the velocity of the first full dorsal flexion decreased 27% ($N=6$, $p<.02$). To test for the possible involvement of central mechanisms, the swim motor program was elicited 10 times (2 min ITI) in isolated brain preparations by electrical stimulation of a nerve root. The duration of the first cycle, as measured by the period of interneuron C2 bursts, increased from 6.2 to 8.0 seconds ($N=7$, $p<.001$). The maximum firing frequency of dorsal flexion neurons, which control the flexion movement, decreased 14% ($N=6$, $p<.01$) in isolated brains. The similarities between behavioral and isolated brain responses suggest that central nervous system modifications are partially responsible for habituation in *Tritonia*.

To test for a change in behavioral threshhold, six animals were stimulated at 2 min intervals. After 20 to 40 stimuli, all animals failed to swim on five consecutive trials, but did swim when a higher intensity stimulus was applied to the same site, indicating that the response failure was due to an increase in behavioral threshhold. Habituation of escape swimming in *Tritonia* therefore involves changes in: number of cycles, cycle duration, flexion velocity, and threshhold.

Recent studies indicate that multiple circuit modifications may mediate habituation in *Tritonia* (Frost, W.N. and P.A. Getting, Soc. Neurosci. Abstr., 1987). We intend to explore the relationship between the multiple circuit modifications and the multiple behavioral changes reported here. Supported by NS17325 and NS07247.

336.5

ADENYLATE CYCLASE ACTIVITY IS REQUIRED FOR LONG-TERM, FACILITATION. D. Dixon* & H.L. Atwood. (SPON: J.M. Wojtowicz). Dept. of Physiology, University of Toronto; Toronto, ONT. M5S 1A8.

Long-term facilitation (LTF) at the crayfish neuromuscular junction is induced by tetanic stimulation and persists for hours. Induction of LTF has been attributed to ionic imbalances produced by the stimulation. These changes in Na^+ and Ca^{++} recover shortly after stimulation, yet facilitation persists for hours. The present study investigates second messenger involvement in LTF. Activators and potentiators of cAMP (IBMX and Forskolin) are effective in producing a long-lasting facilitation similar to the second phase of LTF. Localized pre-synaptic injection of the Walsh Inhibitor (PKI) blocks the second phase of LTF at synapses near the injection site, whereas normal LTF develops at synapses distant from the injection site within the same preparation. Localization of PKI was confirmed by flourescent tagging of the PKI. Adenylate cyclase's (AC) role in LTF is further supported by results using SQ22,536, an AC inhibitor. Localized injection of SQ22,536 blocks the second phase of LTF near the injection site, while synapses distant from the injection show normal LTF. These experiments establish a role for AC activation in producing LTF. Supported by MRC Canada and OGS scholarships.

336.6

INTRACELLULAR CORRELATES OF RESTRAINT-INDUCED MODULATION OF THE CRAYFISH LATERAL GIANT ESCAPE RESPONSE. E.T. Vu and E.B. Krasne. Neuroscience Program, Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Restraint suppresses the lateral giant (LG)-mediated tailflip escape reflex of the crayfish. This suppression involves a tonic form of inhibition descending from thoracic and higher levels of the nerve cord. We are currently investigating the circuit sites and cellular mechanisms underlying this behavioral modulation.

A likely target for extrinsic control is the circuit's command neuron, the LG. We report here two intracellularly observed effects on the LG cell of restraining the animal: (1) A clear decrease in all early phases of the second root-elicited compound postsynaptic potential, which involves inputs from both primary afferents and sensory interneurons and consists mainly of electrical synaptic input to the LG; and (2) A hyperpolarizing shift in the membrane potential, bringing it away from an unchanged firing threshold. We are determining whether the same mechanism underlies both these effects, and whether earlier circuit elements are also modulated.

Supported by N.I.H. grant NS08108 (FK) and a Predoctoral N.S.F. Fellowship (EV).

336.7

OCTOPAMINE MODULATION OF THE SENSORY SYNAPSE IN CRAYFISH LATERAL GIANT ESCAPE REACTION CIRCUIT. J. Bustamante and E.B. Krasne. Department of Psychology, University of California, Los Angeles, CA 90024.

Traumatic events cause a prolonged increase in the probability of lateral giant mediated tailflip escape responses to sudden abdominal stimuli. This behavioral sensitization is associated with increased stimulus sensitivity of first order interneurons of the tailflip reflex circuit. Octopamine mimics these effects.

Here we studied the effect of octopamine on the intracellularly recorded responses of interneuron A. Octopamine (1) increased amplitudes (18-30%), rise times (11-14%), and fall times (40-75%) of unitary EPSPs evoked by stimulation of individual tailfan mechanoreceptors, (2) caused ca 300% increases in "spontaneous" (ambient water-current caused?) firings of sensory root afferents and in size and frequency of "spontaneous" unitary EPSPs, and (3) caused no apparent changes in either resting potential or critical firing level.

Response-to-response variation in unitary EPSP amplitudes, which we believe to reflect variable quantal release, is now being used to evaluate the effects of octopamine on quantal release. Though from parallel studies in Aplysia, altered release would be expected, the altered EPSP rise and fall times seen here may suggest different mechanisms. Supported by C.S.I.C. Fellowship, Spain (JB) and N.I.H. grant NS08108 (FK).

336.8

IDENTIFICATION OF PROTEINS THAT MAY MEDIATE SENSITIZATION IN APLYSIA. A. ESKIN, J.H. BYRNE+, AND K.S. GARCIA*. Bio. Dept., Univ. Houston, +Dept. Neurobiol. & Anat., Univ. Tex. Med. School at Houston, Houston, Tx, 77004.

We investigated whether neuronal proteins of A. californica were altered by procedures that mimic those used to produce long-term sensitization. Samples for 2D-gel electrophoresis were prepared from pleural sensory neurons at the end of treatment periods. We found that exposure to serotonin (5-HT) for 2 hrs increased amino acid incorporation into protein P9 and decreased incorporation into proteins P19 and P20. Forskolin or cAMP analogs mimicked the effects of 5-HT on P9, P19, and P20. A phorbol ester, TPA, had no effect on these proteins, but it increased the amount of label associated with P24. In studies of protein phosphorylation, we found that P9 and P19 are phosphoproteins, but 5-HT did not appear to affect their phosphorylation. However, 5-HT increased phosphorylation of P21 and decreased phosphorylation of P29. Preliminary results indicate that increases in cAMP mimicked the changes in phosphorylation that were produced by 5-HT. The proteins affected by both 5-HT and changes in cAMP may be involved in the induction of short and long-term changes in the nervous system of Aplysia.

336.9

A QUANTITATIVE MODEL FOR THE KINETICS OF cAMP-DEPENDENT PROTEIN KINASE (TYPE II) ACTIVITY: LONG-TERM ACTIVATION OF THE KINASE AND ITS POSSIBLE RELEVANCE TO LEARNING AND MEMORY. J.D. Buxbaum* and Y. Dudai. Dept. of Neurobiology, Weizmann Institute, Rehovot 76100, Israel.

Evidence from *Drosophila* and *Aplysia* implicates persistent activation of the cAMP cascade in memory. Different processes in the cAMP cascade may contribute to its persistent activation, including autophosphorylation of cAMP-dependent protein kinase (PKA), in which the catalytic subunit (C) phosphorylates the regulatory subunit (R). We developed a quantitative model for the interactions between the two PKA subunits, making use of experimentally defined kinetic rate constants and assuming eight functional states for R: four involving dephosphorylated R (R·C, R·cAMP·C, R·cAMP and R), and four similar states involving phosphorylated R. Computer simulations showed that following a transient (several seconds) rise in intracellular cAMP levels, PKA can be activated for tens of minutes under appropriate physiological conditions. This long-term activation of the protein kinase is due in part to the effects of phosphorylation of the regulatory subunit, which decreases the affinity of the regulatory subunit for the catalytic subunit. We have simulated the activation of PKA in *Drosophila* learning and memory mutants, with primary or secondary defects in the cAMP cascade. Our model predicts defective kinetics of PKA in *Ddc*$^-$, *rutabaga*, *dunce* and *turnip* under physiological conditions. Based on these data, combined with previous studies, we suggest that in *Drosophila*, and possibly in other organisms: a) PKA is central to sensitization, habituation, and classical conditioning, and b) PKA may retain information in short-term memory by the process of long-term activation. (Supported by the US-Israel Binational Science Foundation, Jerusalem).

336.10

TYPE II CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE IN DROSOPHILA. D. S. Leonard and M. B. Kennedy. (SPON: R. J. Leonard). Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

We previously reported that the fruit fly Drosophila contains a protein kinase with considerable homology to the rat brain type II CaM kinase (Soc. Neurosci. Abstr. 13, 559, 1987). We now report that cDNA clones coding for both subunits of the rat kinase label homologous sequences in Southern blots of Drosophila genomic DNA. We find that one restriction fragment is labeled by both rat α and β subunit cDNA probes under different low stringency conditions. Additional, non-overlapping fragments are labeled by probes encoding only one of the two rat subunits. Therefore, like the rat kinase, the fly type II CaM kinase may be composed of related subunits encoded by distinct genes.

We have also screened fly head cDNA libraries with probes made from rat kinase α and β subunit cDNAs. A clone was recovered which hybridizes to probes made from N-terminal and C-terminal portions of both rat subunits. Bulleit et al. recently pointed out that N-terminal portions of both rat subunits include highly conserved kinase and calmodulin binding domains, whereas C-terminal portions contain sequences unique to the type II CaM kinase (Neuron 1, 63-72, 1988). Thus, this clone appears to encode a large portion of one of the fly kinase subunits. Sequencing is now underway. Supported by NIH.

336.11

LIGHT PAIRED WITH ACTIVATORS OF PROTEIN KINASE C PRODUCES SHORT-AND-LONG-TERM ENHANCEMENT OF LIGHT RESPONSES OF LATERAL B-PHOTORECEPTORS IN HERMISSENDA. T. Crow and J. Forrester*. Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

Previous results have indicated that protein kinase C (PKC) may play a role in the induction of short-term plasticity in type-B photoreceptors. We now present evidence that light paired with activation of PKC is sufficient to produce both a short-term (1hr) nonassociative and long-term (24 hrs) associative enhancement of light-evoked generator potentials recorded from lateral B photoreceptors. A light step (5 min) was paired with the direct injection of PKC activators such as 1 uM TPA or 1 uM OAG onto the exposed nervous system of otherwise intact Hermissenda. Both activators of PKC produced an enhancement of the peak amplitude of the generator potential ($\bar{X}$=42.8mV) and the amplitude at light offset ($\bar{X}$=38mV). Preparations receiving control injections exhibited normal light responses ($\bar{X}$=40.1mV and $\bar{X}$=26.1mV, respectively). The application of PKC activators when paired with light produced a pairing specific long-term change in the amplitude of the generator potential (peak amplitude; $\bar{X}$=46 mV; amplitude at light offset; $\bar{X}$=29.2mV). Unpaired controls exhibited smaller responses when tested 24 hrs after unpaired light and PKC activators ($\bar{X}$=43.3mV; $\bar{X}$=25.7mV). These results indicate that light paired with activation of PKC can mimic some of the electrophysiological effects of pairing light and 5-HT on changes in the light responses of identified B photoreceptors in Hermissenda.

336.12

PROTEIN KINASE C INHIBITION PREVENTS SHORT-TERM BUT NOT LONG-TERM LIGHT-5-HT-INDUCED ENHANCEMENT OF GENERATOR POTENTIALS IN HERMISSENDA B-PHOTORECEPTORS. J. Forrester* and T. Crow (SPON: A. Moller). Dept. of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

Light paired with direct application of 5-HT to the exposed nervous system of otherwise intact Hermissenda produces both short-and-long-term changes in the amplitude of light-evoked generator potentials recorded from identified photoreceptors. Previous results have suggested that activation of protein kinase C (PKC) may contribute to the induction of plasticity produced by pairing a neuromodulator with activity in B-photoreceptors. We now present evidence that an inhibitor of PKC produces different effects on short-and-long-term plasticity. Application of the PKC inhibitor H-7 (1 μM) (Hidaka, 1984) blocked the short-term enhancement of light-evoked generator potentials produced by light and 5-HT (mean control peak amplitude=41.4mV, mean amplitude at light offset=27mV; H-7 mean peak amplitude=39.2mV, mean amplitude at light offset=26.5mV; H-7 + 5-HT mean peak amplitude=38.2mV, mean amplitude at light offset=26.8mV). Light responses following the application of 5-HT without H-7 were enhanced (mean peak=43.6mV, mean amplitude at light offset =29.7mV). In contrast to the short-term effects, H-7 did not block the long-term (24 hrs) enhancement of generator potentials produced by pairing light and 5-HT (mean peak amplitude=43.3mV, mean amplitude at light offset=29.8mV). These results suggest that other messenger systems may contribute to the long-term enhancement of light responses produced by light and 5-HT.

336.13

5-HT MODULATES LIGHT-EVOKED CURRENTS AND DISCRETE WAVES IN B-PHOTORECEPTORS OF HERMISSENDA. T.Pengidore* and T. Crow (SPON: A.J. Martinez). Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

In Hermissenda type-B photoreceptors the intervals between light-evoked discrete waves follow an exponential frequency distribution. Since previous research has shown that 5-HT can modulate light responses we have examined the effects of the neuromodulator 5-HT on the interwave interval distributions of light-evoked discrete waves in axotomized type B-photoreceptors. Dim illumination evoked discrete waves that conformed to an exponential interval distribution. Following bath application of .1mM or .01mM 5-HT, dim illumination evoked a decrease in the intervals between discrete waves and the intervals again followed an exponential frequency distribution. The frequency of light-evoked discrete waves calculated from the mean of the interval distribution showed a 32% increase following the bath application of 5-HT. Chi-square analysis of the fit of the observed distribution to the expected exponential distribution revealed no significant deviation ($P>.05$). Application of 5-HT in the dark did not produce a significant change in the frequency of spontaneously occurring discrete waves. The analysis of light-evoked currents from photoreceptors voltage-clamped at their dark-adapted resting potential followed an exponential interval distribution. The average increase in the frequency of light-evoked currents in the presence of 5-HT was 28%. The analysis of discrete waves may provide insights into the action of second-messenger systems activated by neuromodulators in Hermissenda.

336.14

PROTEIN DEFICIENT DIET RETARDS LEARNING IN APLYSIA. J.M. Flinn, S. Kurtz*, D. Royall*, Department of Psychology, George Mason University, Fairfax, VA 22030.

The development of learning in juvenile aplysia takes place in clearly defined stages, with sensitization appearing during stage 12 (Rankin and Carew, 1987). To explore the effect of protein deprivation on learning, we raised animals from age 50 days to age 110+ days on two different algae, gracillaria and enteromorpha, which had different protein content. All animals were obtained from the Aplysia Mariculture Facility in Woods Hole and were full siblings. Sensitization was examined by videotaping and analyzing escape behavior following tail shock. Threshold shocks were determined for all animals. Two weak stimuli at 1.5 x threshold current were administered two mins apart, followed either by a strong sensitizing shock (4 x threshold current) or by a weak shock. Two additional test shocks were given five mins later. Animals (N=20) raised on a gracillaria whose protein content at the beginning of the study was 33% (dry weight) did show sensitization. The measure used was the number of steps taken in the two mins after each test shock. Animals receiving a sensitizing shock took sig. more steps after the sensitizing shock than the controls ($p<.006$). Animals (N=19) raised on an enteromorpha, whose protein content was 16%, did not show a significant increase in steps. These results indicate that protein deprivation during development interferes with the acquisition of sensitization in juvenile aplysia.

336.15

MODIFIABLE BEHAVIOR AND AGE-SENSITIVITY MAY BE RELATED IN APLYSIA. B. Hallahan*, B. Peretz & T. Skinner*. Dept. Physiol. & Biophys., Univ. of Ky., Lexington, KY 40536.

Peretz et al. (1984) proposed that the modifiable gill withdrawal reflex (GWR) is more age-sensitive than the periodic respiratory gill pumping movements (GPM). We based this on the age-impaired function of neuron L_7 and the age-invariant function of LDG_1; L_7 and LDG_1 are major contributors of the GWR and GPM respectively. To determine the applicability of this proposal to freely moving animals, we examined the GWR and GPM in the same mature (ca 150 days) and old (ca 250 days) animals. The GWR was elicited by artifical seawater (ASW) jets of 0.4 to 8.0 g/cm^2 to the siphon. The GWR duration was reduced in old animals, $F(4,96) = 2.51$, $p<0.05$. The GWR habituated faster in old than in mature animals to repetitive jets of 2.0 g/cm^2, $F(9,198) = 2.74$, $p<0.005$. Elevated GPM rates were elicited by placing the animals in ASW from pH 7.8 (normal ASW) to 3.0 for 5 min intervals. The GPM rates were the same in both groups. The rate did not habituate to repetitive exposure to pH 5.0. When elicited together, to test if the GWR and GPM interact, the GPM rates at pH 7.8 and 5.0 were unaffected by siphon stimulation, but the GWR duration was less in pH 5.0 than in pH 7.8. In these animals the GWR and GPM express the age effects of their respective substrates. The GWR is more modifiable by sensory input and age than the GPM, and this suggests that behavioral modifiability and vulnerability to age may be related.

336.16

PERSISTENCE OF ULTRASTRUCTURAL CHANGES AT IDENTIFIED SENSORY NEURON SYNAPSES DURING LONG-TERM SENSITIZATION IN APLYSIA. M. Chen and C.H. Bailey. Ctr. for Neurobiol. & Behav., Dept. of Anat. and Cell Biol., Neurol., & Psychiat., Columbia P&S, and NYSPI, NY, NY 10032.

The time course of changes in active zone morphology at identified sensory neuron synapses was examined at different intervals following long-term sensitization of the gill-withdrawal reflex. HRP-labeled varicosities in 20 μm slab-thick sections were re-embedded, thin sectioned, and analyzed through a blind procedure; 693 sensory neuron varicosities taken from 14 animals were treated in this fashion. As reported in an earlier study, we have found that long-term sensitized animals examined within 48 hrs following the completion of training demonstrate an increase in the incidence (0.395 ± 0.03 S.E.M. vs. 0.21 ± 0.02), length ($0.51\,\mu m \pm 0.02$ vs. $0.24\,\mu m \pm 0.01$) and vesicle complement (4.2 ± 0.19 vs. 1.65 ± 0.15) of sensory neuron active zones compared to control animals. The increase in active zone number is maintained at 1 week (0.386 ± 0.02, N=3 vs. 0.22 ± 0.02, N=2, $t=4.9$, $p < .05$) and is only partially reversed at the end of three weeks (0.27 ± 0.01, N=3 vs. 0.19 ± 0.01, N=2). In contrast, the increase in active zone size and vesicle complement is not present after 24-48 hrs. The relative permanence of changes in active zone number and their similarity in time course to both changes in varicosity number as well as the duration of the memory strengthen the evidence that alterations in the number of sensory neuron synapses may contribute to the retention of long-term sensitization.

336.17

MORPHOLOGICAL EVIDENCE THAT COMPETITION FOR POSTSYNAPTIC SPACE AND SEROTONIN STIMULATION CAN REGULATE THE GROWTH OF APLYSIA SENSORY NEURONS IN CELL CULTURE. D.L. Glanzman, E.R. Kandel and S. Schacher. Howard Hughes Med. Instit., Ctr. for Neurobiol. & Behav., Columbia P&S, and NYSPI, New York, NY 10032.

We have examined the relationship between morphological changes and long-term synaptic changes for Aplysia sensorimotor synapses in culture. After assessing the strengths of synaptic connections between sensory neurons and motor cell L7 electrophysiologically, the processes of the sensory neurons were visualized via video fluorescence microscopy. Our findings suggest that the neurites which mediate synaptic contact between a sensory neuron and its postsynaptic target are not "hard-wired." Rather, they can be regulated by modulatory transmitters, such as 5-HT, and by competitive interactions with the processes of other sensory neurons. Application of 5-HT to sensorimotor cultures causes growth of the sensory processes, particularly those contacting L7's major neurite. Also, in mature cell cultures different neurites of L7 appear to be occupied by processes from different sensory neurons. Only the major neurite of L7 is regularly colonized by processes from different sensory neurons and, in these instances, the different processes are segregated onto different regions of the neurite. These findings provide structural correlates for the functional plasticity which characterizes this synapse, as well as evidence for a previously unsuspected competitive mechanism regulating the formation of connections between Aplysia sensory and motor neurons.

INVERTEBRATE LEARNING AND BEHAVIOR III

337.1

NEW APPARATUS FOR OPTICAL RECORDING OF NEURON ACTIVITY. J.-Y. Wu,* V. Pantani,* H. Abildgaard,* L.B. Cohen, D.M. Senseman, D. Schiminovich,* A.I. Cohen,* C. Xiao,* H.-P. Hopp,* and J.A. London. Dept. of Physiology, Yale Univ. Sch. of Med., New Haven, CT, 06510, Div. of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX, 78285.

In an attempt to facilitate the implementation and increase the usefulness of optical recording methods, we are in the process of introducing a new photodiode array, associated amplifiers, and computer system. The photodiode array, made by Centronics Ltd., has 464 elements (instead of the present 144) to provide better spatial resolution. The amplifiers are expected to be similar in electronic characteristics to those presently in use but would be made with either surface mount or hybrid technology (hopefully much less expensive). The new computer system is a 32 bit computer using the VME bus and a 68020 CPU. This Motorola, VERSADOS system is both easier to program and it is multi-user. The computer includes a 16 bit, 400 kHz, 512 channel, analog-to-digital converter. While we hope that the new apparatus will be an improvement on the old, the main limitation on the usefulness of optical recording methods will still be the size of optical signals from voltage or ion sensitive dyes.

337.2

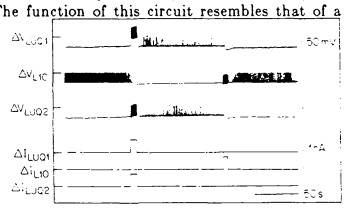
SMALL NETWORKS OF ADAPTIVE ELEMENTS THAT REFLECT THE PROPERTIES OF NEURONS IN *APLYSIA* EXHIBIT HIGHER-ORDER FEATURES OF CLASSICAL CONDITIONING. J.H. Byrne, D. Buonomano*, I. Corcos*, S. Patel* & D.A. Baxter. Dept. of Neurobiol. & Anat., The Univ. of Tex. Med. Sch., Houston, TX 77225.

Previously, we developed a single-cell mathematical model of the sensory neurons in *Aplysia* (Gingrich & Byrne, *J Neurophysiol* 53:652, 1985; *J Neurophysiol* 57:1705, 1987). This single-cell model accurately simulated many aspects of empirically observed neuronal plasticity that are believed to be cellular correlates of simple forms of nonassociative and associative learning. The present study extends our analysis by incorporating this single-cell model into small networks of adaptive elements which reflect the neuronal properties and connectivity patterns in *Aplysia*. An initial network contained two sensory neurons (SNs) both of which excited a single facilitatory interneuron (FN), that feeds back onto the SNs. An assumed property of the FN was that its output accommodated as a result of its activation (Hawkins & Kandel, *Psychol Rev* 91:375, 1984). Simulations from this network exhibited 2nd order conditioning but not asymptotic blocking when the original model of the SN was used. Both 2nd order conditioning and asymptotic blocking were simulated by modifying the model such that: 1) the synaptic strength of the conditioned SN (CS+ cell) was at least twice as large as an unconditioned SN (CS- cell), 2) the time required for complete accommodation of the FN was less then the minimum ISI necessary for conditioning in the SNs, and 3) the Ca^{2+} levels within the SNs first must surpass a threshold value before associative plasticity can occur. We also investigated how the incorporation of additional interneurons that recieve excitatory input from, and feed back to inhibit the SNs, would alter the above constraints and possibly make the network more robust. Various models proved functional, but each required specific assumptions. Supported by grant AFOSR 87-020.

337.3

Circuits with Bistable Outputs Constructed from Identified Neurons. D. Kleinfeld, G. F. Raccuia* & H. J. Chiel. AT&T Bell Laboratories, NJ 07974.

We have used identified neurons from the abdominal ganglion of the mollusc *Aplysia* to construct two circuits *in vitro*.[1] These circuits exhibit bistable outputs. The first circuit consisted of co-cultured L7 and L12 neurons; these formed reciprocal, excitatory connections. In one stable state both cells were quiescent and in the other stable state both cells were firing. A pulse of depolarizing current to either cell caused the circuit to make a transition from the quiescent to the active state. The second circuit consisted of an L10 neuron co-cultured with LUQ neurons. We blocked the calcium activated potassium channels in the L10 and the LUQs with charybdotoxin; this suppressed the normal bursting output of these cells. In one such circuit the L10 formed reciprocal, inhibitory connections with two LUQs; the LUQs were electrically coupled (Fig.). In one stable state the L10 was active and the LUQs were quiescent and in the other stable state the L10 was quiescent and the LUQs were active. Injection of a depolarizing pulse of current into the quiescent neuron(s) caused them to fire persistently, thus inhibiting the previously active neuron(s). The function of this circuit resembles that of a "flip-flop". The essential features of the bistable behavior for both circuits are believed to arise from the non-linearity of the input-output relation for the neurons and the (approximate) symmetry of the synaptic connections.



1 - S. Schacher & E. Proshansky (1983) *J. Neurosci.* 3:2403.

337.4

A CELLULAR ANALYSIS OF TAIL-SHOCK INDUCED INHIBITION IN THE SIPHON WITHDRAWAL REFLEX OF *APLYSIA* W.G. Wright*, E.A. Marcus, H. Thaker* and T.J. Carew, (SPON: N. Donegan), Depts. of Psychology & Biology Yale Univ, New Haven, CT 06520

Tail shock, which is known to produce facilitation of the siphon withdrawal reflex in *Aplysia*, has also recently been shown to produce behavioral inhibition of the reflex (Marcus et al, 1987; Mackey et al, 1987; Krontiris-Litowitz et al, 1987). The present study was directed at a cellular analysis of this tail-shock induced inhibition.

To mimic the behavioral studies of Marcus et al (1987), which showed a transient inhibition of siphon withdrawal 90 secs after multiple tail shocks, we used a semi-intact preparation (abdominal ganglion connected to the tail and siphon via the intact CNS) to examine the effects of tail shocks on: 1) complex EPSPs in siphon motor neurons (elicited by water-jet stimuli to the siphon); and 2) monosynaptic EPSPs (elicited by intracellular sensory neuron activation). Complex and monosynaptic EPSPs were tested twice at a non-decrementing (10 min) ISI to assess baseline. A TAIL SHOCK group (N=9) then received 4 strong shocks; a CONTROL group (N=7) received no shock. Test EPSPs were expressed as a percent of mean baseline EPSPs. The TAIL SHOCK group showed significant inhibition of the complex EPSP 90 sec after shock, (med=38%) compared both to baseline ($p<.01$) and to CONTROL (med=99%; $p<.02$). The complex EPSP recovered 10 min after shock (med=83%): it was elevated compared to the 90 sec test ($p<.02$) and was no longer different from CONTROL (med=81%). The effect of tail shock on the monosynaptic EPSP (tested at 2 min; med=136%) was significantly different from the effect on the complex EPSP at 90 sec ($p<.05$).

These results show that tail shock can produce transient inhibition of complex synaptic input to siphon motor neurons with a time course parallel to behavioral inhibition.

337.5

DEVELOPMENTAL DISSOCIATION OF DISHABITUATION AND SENSITIZATION IN THE TAIL-WITHDRAWAL REFLEX OF *APLYSIA* M. Stopfer & T.J. Carew, Dept. Psychol., Yale Univ., New Haven, CT 06520.

Dishabituation and sensitization have previously been developmentally dissociated on both behavioral and cellular levels in the siphon withdrawal reflex of *Aplysia*; dishabituation emerges early (in Stage 10) whereas sensitization emerges about 60 days later (in Stage 12) (Rankin & Carew, 1988; Nolen & Carew, 1988). To examine whether this dissociation is a general feature of development in *Aplysia*, in this study we examined another response system, tail-withdrawal.

Stage 11 animals sequentially received two types of training, so that each animal served as its own control. First, SENSITIZATION TRAINING: 3 weak water-jet pre-test stimuli to the tail at a non-decrementing (5 min) ISI, followed by either tail shock (TS, N=5) or head shock (HS, N=5), followed by 3 test stimuli (ISI = 5 min). Second, DISHABITUATION TRAINING: 15 pre-test stimuli at an habituating ISI (5 sec), followed by tail or head shock, followed by 5 test stimuli (ISI = 5 sec). For both groups, (comparing percent change in test vs pre-test) no sensitization was observed; in fact, there was a trend towards inhibition (TS $\bar{x}$ = -36.0%; HS $\bar{x}$ = -0.2%). In contrast, both groups exhibited significant dishabituation (TS $\bar{x}$ = +29.4%, $p<.01$; HS $\bar{x}$ = +53.4%, $p<.05$). Preliminary evidence from similar experiments in older animals suggests that, as in siphon withdrawal, sensitization of tail-withdrawal emerges during Stage 12.

In conclusion, dishabituation and sensitization of tail withdrawal can be developmentally dissociated in juvenile *Aplysia*. Since the tail-withdrawal reflex is well suited for a cellular analysis (Walters et al, 1983), it will now be of interest to explore the ontogeny of dishabituation and sensitization in this system on a mechanistic level.

337.6

DEVELOPMENT OF TAIL SENSORY NEURONS IN THE PLEURAL GANGLIA OF *APLYSIA* E.A. Marcus, G. Schuerman*, M. Stopfer, and T.J. Carew, Depts. of Biol. and Psych., Yale Univ., New Haven, CT 06520

Tail sensory neurons in the VC cluster of the pleural ganglion of *Aplysia* are important sites of plasticity underlying different forms of learning in the tail-withdrawal reflex (Walters et al, 1983; Scholz and Byrne, 1987). Stopfer and Carew (1988) have recently shown that dishabituation and sensitization emerge differentially in this reflex. A first step in analyzing the cellular mechanisms underlying this developmental dissociation is to define and characterize the tail sensory neurons in juvenile animals.

To examine the development of tail sensory cells, we backfilled nerve P9 (which contains axons of tail sensory and motor neurons) with Ni^{++}-lysine in 3 juvenile stages (11, E12, and L12) and 3 early adult substages (13A, 13B and 13C). As early as Stage 11, filled cells appeared in an identifiable cluster. We found that there was no increase in the number of filled cells during juvenile development (Stage 11 $\bar{x}$ number of cells=10.5; Stage 13C $\bar{x}$=12.5). However, there was a significant increase in cell diameter ($F=136$, $p<.001$), ranging from $\bar{x}$=10 μ in Stage 11 to $\bar{x}$=42 μ in Stage 13C. This increase in mean diameter reflected a selective increase in the number of large cells, and a concommitant decrease in the number of small cells, resulting in a transition from a unimodal distribution of small cells in Stage 11, to a bimodal distribution of small and large cells in Stage 13C. The gradual nature of this transition and the lack of an increase in total cell number suggests that the emergence of the subpopulation of large cells in Stage 13C is due to the growth of previously existing small cells.

Having identified a candidate population of tail sensory neurons early in development, we are now characterizing their biophysical properties to examine the development of neuromodulation in the tail withdrawal circuit.

337.7

INHIBITORY INTERNEURON PRODUCES HETEROSYNAPTIC INHIBITION OF THE SENSORY-MOTOR CONNECTION MEDIATING THE TAIL WITHDRAWAL REFLEX OF APLYSIA. D. Buonomano*, L.J. Cleary & J.H. Byrne (SPON: J. Wood). Dept. of Neurobiology and Anatomy, Univ. of Texas Med. School, Houston, TX 77225.

Modulation of synaptic efficacy between the sensory (SN) and motor neurons (MN) mediating the tail withdrawal reflex in Aplysia has been correlated with modifications of that behavior. We have identified an interneuron (InhN) that inhibits transmission at this synapse. Action potentials were elicited in a SN at 1 min intervals, producing monosynaptic EPSPs in the MN. The InhN was stimulated at high frequency just before and during the 4th spike in the series. When the InhN produced a hyperpolarization of at least 2 mV in the MN, the amplitudes of the 3rd, 4th and 5th EPSPs averaged 0.6, 0.3 and 0.5, respectively, when normalized to the 1st EPSP (n=5). This example of heterosynaptic inhibition was due, at least in part, to membrane changes in the postsynaptic cell which were associated with an increased membrane input conductance. We cannot rule out a presynaptic contribution to the inhibition, however, since InhNs also hyperpolarized SNs. These effects are probably monosynaptic since the hyperpolarization of both SNs and MNs persisted in bath solutions containing high divalent cations.

InhNs may play a role in the response to tail stimulation. They receive strong excitatory synaptic input from peripheral nerves P8 and P9, and they have axons in P9. Moreover, in one semi-intact preparation the InhN responded to mechanical stimulation of the tail with a short burst of spikes. InhNs also receive excitatory input from SNs. High frequency stimulation of SNs increased the frequency of spontaneous EPSPs in InhNs, but occasionally a single spike in a SN directly elicited a discrete EPSP. Like P116, another inhibitory pleural interneuron (Mackey et al., 1987), InhNs have axons in the pleural-abdominal connective, but they do not appear to contain the same transmitter, FMRFamide.

At present, the function of inhibitory interneurons in the response to tail stimulation is not well understood. However, these neurons could contribute to the hyperpolarization of SNs that is produced by stimulation of regions of the tail that are outside of the neuron's receptive field. They could also contribute to the transient behavioral inhibition that is produced by sensitizing stimulation of the tail.

337.8

SEROTONERGIC VARICOSITIES MAKE APPARENT SYNAPTIC CONTACTS WITH PLEURAL SENSORY NEURONS OF APLYSIA. Z. Zhang*, L.J. Cleary, D.W. Marshak, J.H. Byrne, Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77225.

The mechanisms within sensory neurons contributing to sensitization of defensive reflexes in Aplysia can be activated by the neurotransmitter serotonin. Varicosities containing serotonin are distributed among the cell bodies of sensory neurons in the pleural ganglion. To further characterize the morphology of serotonergic varicosities and their contacts with sensory neurons, we used the immunoperoxidase technique (Eldred et al., 1983) with correlated light (LM) and electron microscopy (EM).

In the LM, we observed numerous varicosities surrounding somata of sensory neurons. In favorable sections, labeling of fibers surrounding the axon hillock could also be observed. Varicosities (n=) were classified into three groups based on size: small (less than 1 μm), medium (1 to 3.5 μm), and large (3.5 to 6 μm). Individual axons can form varicosities of all three types, but branch only at those of large size.

Because the somata of sensory neurons are encapsulated by glia, it is necessary to confirm that reactive varicosities directly contact membranes of sensory neurons. In the EM, we observed these contacts on both the soma and the axon hillock. Since the electron dense reaction product obscured presynaptic ultrastructure, and there are not obvious postsynaptic specializations in Aplysia, we must assume that these contacts are synaptic structures. After measuring the shortest diameter of each varicosity (0.1 to 0.9 μm; n= 28), it appears that only small varicosities make contacts. In addition, there appear to be two types of synaptic morphologies: flat, in which the area of contact is small, and invaginated, in which the serotonergic profile is nearly surrounded by the sensory neuron.

These results suggest that under the appropriate physiological conditions, serotonin is released from varicosities directly onto perikarya of sensory neurons eliciting the cellular changes correlated with sensitization. That there are two types of synaptic contacts raises the possibility that this synapse is also a site of plasticity. An important future task is to identify the neurons that give rise to these varicosities, so that their role in behavioral modifications can be characterized more completely.

337.9

SEROTONIN BROADENS ACTION POTENTIALS IN BOTH SOMATA AND AXONS OF PLEURAL SENSORY NEURONS IN APLYSIA. M. Hammer*†, L.J. Cleary and J.H. Byrne, Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77225 and †Institut für Neurobiologie, Freie Universität, 1000 Berlin 33 FRG.

The enhanced release of transmitter from synapses between sensory and motor neurons is a cellular mechanism underlying non-associative and associative learning in Aplysia. The enhancement is believed to be due, at least in part, to broadening of action potentials in the sensory neurons. While broadening is readily observed in recordings from the cell soma, parallel broadening in the synaptic terminals has only been inferred. Therefore, an important test of this model is the direct demonstration that action potentials are broadened at or near the terminals. In the pleural ganglion, the somata of sensory neurons are located 2-3 mm away from their synaptic contacts with motor neurons in the pedal ganglion. Therefore, by recording directly from the axons of sensory neurons in the pedal ganglion, the kinetics of action potentials near synapses can be compared with those far away in the soma.

Simultaneous recordings were made from sensory and motor neuron somata and from sensory neuron axons. Application of 50-300 μM serotonin to the bath broadened action potentials in both the soma and axon and enhanced the EPSP in the motor neuron. The degree of broadening is more apparent in the soma than in the axon, however. The same effects were observed when somata of the sensory neurons were separated from distal axons by cutting the pleural-pedal connective. This suggests that modulation of membrane properties in the soma is not necessary to produce broadening in the axon.

To show that the site of axon recording was electrically close to the site of transmitter release, we altered the membrane potential from which the action potential was generated. By hyperpolarizing the axon 10 mV, we decreased the amplitude of the EPSP by an average of 25%. In contrast, hyperpolarizing the soma 10 mV did not affect the amplitude of the EPSP. An average decrease of 6% was due to homosynaptic depression. Thus, our recording sites in the axon were electrically close to synaptic terminals, whereas those in the soma were relatively far.

These experiments demonstrate for the first time that spikes near the terminals of sensory neurons in Aplysia are broadened by a neurotransmitter that alters synaptic transmission. These results support the hypothesis that spike broadening is a cellular mechanism underlying learning. Further experiments will be required to determine the quantitative extent to which this mechanism accounts for the observed changes in synaptic efficacy.

337.10

AN IDENTIFIED FMRFamide-IMMUNOREACTIVE NEURON PRODUCES PRESYNAPTIC INHIBITION OF THE SIPHON SENSORY NEURONS IN APLYSIA. R.D. Hawkins and S.A. Small* (SPON: D.W. Burmeister). Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, & NYSPI, New York, NY 10032.

Noxious stimuli such as tail shock produce transient inhibition as well as longer-lasting sensitization of the Aplysia gill- and siphon-withdrawal reflex. The inhibition is due in part to presynaptic inhibition of the siphon sensory neurons which can be mimicked by application of the peptide FMRFamide. To search for neurons which participate in mediating the inhibition, we combined FMRFamide immunofluorescence with fluorescent dye backfilling from the abdominal ganglion (the location of the siphon sensory cells). This technique revealed a single cell in the left pleural ganglion, which we have named LPL16. LPL16 is excited by strong tactile stimulation to the entire body surface, and fires a phasic burst of spikes in response to tail shock. Intracellular stimulation of LPL16 with a similar burst produces inhibition of the EPSP from a siphon sensory neuron to a motor neuron ganglion (average decrease = 52%, $p < .01$). Such stimulation also produces narrowing of the action potential in the sensory neuron in the presence of 100 mM TEA (average decrease = 26%, $p < .01$), indicating that the inhibition is in part presynaptic.

These and previous results show that tail shock excites two different populations of modulatory neurons, including serotonergic facilitatory neurons (CB1) and FMRFamide inhibitory neurons (LPL16). Further characterization of these neurons may help elucidate the organization of neuromodulation in Aplysia.

337.11

ACTIVITY-DEPENDENT ENHANCEMENT OF PRESYNAPTIC INHIBITION IN APLYSIA SENSORY NEURONS. S.A. Small*, R.D. Hawkins and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, & NYSPI, New York, NY 10032.

The gill- and siphon-withdrawal reflex of Aplysia undergoes transient inhibition and longer-lasting sensitization following a noxious stimulus such as tail shock. The inhibition is due in part to presynaptic inhibition of the siphon sensory neurons by FMRFamide acting through arachidonic acid, and the sensitization is due in part to presynaptic facilitation by serotonin acting through cAMP. The facilitation undergoes activity-dependent enhancement contributing to classical conditioning of the withdrawal reflex. To test whether the inhibition also shows activity dependence, we puffed FMRFamide onto the cell body of a siphon sensory neuron either 0.5 seconds ("paired") or 5.5 seconds ("unpaired") after a brief train of action potentials in the sensory neuron. Paired training produced significantly larger and longer-lasting inhibition of the EPSP from the sensory neuron to a motor neuron than either unpaired training (mean decrease = 38% vs. 21%, $p < .02$) or training with FMRFamide alone (28% vs. 17%, $p < .05$). Paired training also produced significantly larger and longer-lasting narrowing of the action potential in the sensory neuron in 100 mM TEA than unpaired training (29% vs. 23%, $p < .05$ one-tail), suggesting that the effect is presynaptic.

These results indicate that activity-dependent enhancement occurs for inhibition as well as facilitation of the siphon sensory neurons, and suggest that activity-dependence may be a widespread associative cellular mechanism.

337.12

CONOPRESSIN G AND S SUPPRESS THE GILL WITHDRAWAL REFLEX IN AN IN VITRO PREPARATION OF APLYSIA CALIFORNICA. M. Martinez-Padron* and K. Lukowiak. Dept. of Medical Physiology, University of Calgary, Alberta, Canada, T2N 4N1.

Two novel peptides of the vasopressin-oxytocin family, conopressin G and S, have been isolated from the venom of fish-hunting snails, genus Conus (Cruz et al.,1987). Arginine-vasotocin (AVT), another peptide of this family, mimics the suppressed behavioral state seen in Aplysia. This state is characterized by a weaker gill withdrawal reflex evoked by siphon stimulation and a faster rate of gill reflex habituation (Thornhill et al., 1981). In addition, an AVT-like peptide is present in the Aplysia central nervous system (Moore et al.1981). In this study we have examined the effect of both conopressin G and S on gill behaviors using an in vitro gill, siphon, mantle and abdominal ganglion preparation. Superfusion of the abdominal ganglion with Conopressin G or S (1-10 μM) resulted in a decrease in the amplitude of the gill withdrawal reflex evoked by tactile stimulation of the siphon and a decrease in the synaptic input to central gill motorneurons. In addition, these peptides reduced the efficacy of the central gill motorneurons to produce a gill withdrawal response. Finally, conopressin G and S, greatly increase the frequency of spontaneous gill movements.

337.13

COMPARISON OF DISHABITUATION AND SENSITIZATION OF THE GILL-WITHDRAWAL REFLEX IN APLYSIA. S.L. Mackey*, N. Lalevic*, R.D. Hawkins and E.R. Kandel (SPON: L.P. Rowland). Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, & NYSPI, New York, NY 10032.

Noxious stimuli such as tail or mantle shocks produce an increase in the strength of the Aplysia gill- and siphon-withdrawal reflex if it has been habituated ("dishabituation") or if it is rested ("sensitization"). Marcus et al. (1987) have reported that there is dishabituation 1-2 minutes after the shock, but sensitization does not appear until 10-20 minutes after the shock. Looking at a different response measure (amplitude of gill withdrawal instead of duration of siphon withdrawal) in an isolated mantle organ preparation, we also found that there is dishabituation but no sensitization 2.5 min after a shock to the mantle (Med = 1137% vs. 91% of preshock, $p < .01$). However, dishabituation and sensitization both occur 12.5 minutes after the shock (693% vs. 450%, n.s.).

In a preliminary cellular analysis, we have found that shock produces inhibition followed by facilitation of the monosynaptic EPSP from a siphon sensory neuron to a motor neuron. These effects are approximately the same whether the EPSP is depressed (10 pretests) or rested (2 pretests). NDGA, a blocker of arachidonic acid metabolism, partially blocks the inhibition, revealing an earlier onset of facilitation. These results suggest that some of the differences between dishabituation and sensitization could be due to sculpting of the facilitation by a competing inhibitory process.

337.14

GABA MEDIATION OF VISUAL-VESTIBULAR INTERACTION IN HERMISSENDA M.L. Anderson* and D.L. Alkon. LMCN, NINCDS-NIH Bethesda, MD 20892.

Convergence of CS and UCS pathways activated during associative conditioning of the nudibranch mollusc Hermissenda involves inhibition of type B photoreceptors by hair cells caudally located in statocysts. This inhibition, manifest as IPSP's elicited in type B cells by stimulation of presynaptic hair cells, was unaffected by the bath application of agents known to act as either agonists or antagonists of identified neurotransmitters (NE, ACh, 5-HT, DA, Glu). However, the GABA-A antagonist bicuculline MeBr consistently blocked type B cell hyperpolarization induced by hair cell impulses. GABA and the GABA-A agonist muscimol hyperpolarized and altered the I-V relation of both intact Type B cells and cells isolated from synaptic interactions (axotomized). Muscimol applied in concentrations ranging from 75 uM to 450 uM hyperpolarized B cells 2.5 mV to 10.2 mV (n=5). N-propyl acetate (NPA) enhanced both the hair cell inhibition of type B cells (n=5) and the hyperpolarization of B cells in response to bath applied GABA without hyperpolarizing axotomized type B cells when applied alone (n=4). Voltage clamp studies implicate a GABA-A mediated increase in inward Cl- current during the hair cell inhibition of type B cells and a much more slowly developing, but persistent decrease of K+ currents (n=4). Both of these GABA effects may be important for generating persistent changes in ionic currents after Hermissenda conditioning.

337.15

A QUANTITATIVE MODEL OF BIOPHYSICAL MECHANISMS UNDERLYING TEMPORAL SPECIFICITY OF ASSOCIATIVE LEARNING IN *HERMISSENDA*. C.Chen*, C.Koch and D.L.Alkon (SPON: H.A.Lester). Dept. of Computation and Neural Systems, 216-76, California Institute of Technology, Pasadena, CA 91125 and Lab of Cellular and Molecular Neurobiology, NIH-NINCDS, Rockville, MD 20852.

Hermissenda is able to store predictive relationships between events if they occur in a temporally contigeous manner. This type of associative learning may be mediated by cellular interactions between neurons and be stored by neuromodulation of certain ionic channels. A quantitative model simulated on a parallel computor of the hypercube family was built to relate the temporal specificity at the behavioral level to the underlying biophysical mechanisms at cellular and molecular levels. The network consists of sensory neurons (photoreceptors and haircells), convergent loci on photoreceptors and motor neurons. Seven membrane conductances were modeled by Hodgkin-Huxley-like equations to predict the receptor potential and excitability of Type B photoreceptors. Intracellular calcium was computed by considering light-induced intracellular release, voltage-gated influx, buffer, diffusion and pumps. Haircells fire with rotation and indirectly cause calcium influx on B-photoreceptors through excitatory synapses. Phototransduction is known to cleave PIP2, thereby increasing diacylglycerol (DG). The excitability of the convergent site, located on the photoreceptor somata and the post-synaptic region on the axon, is modulated by the activation of protein kinase C, which depends on the elevation of calcium and DG. The model predicts that repetitive association of light (CS) and rotation (US) with 1 second delay will optimally increase the excitability of B-photoreceptor, which eventually can excite the motor neurons involved in foot contraction in absence of haircell inputs.

337.16

MOLLUSCAN OLFACTORY INTERNEURONS: STRUCTURAL STUDIES AND MODULATION BY INTRINSIC PEPTIDES AND EXTRINSIC AMINES. A. Gelperin, J. Flores*, P.G.Sokolove[1], D.W. Tank, and S.Curtis*[2] Molecular Biophysics Research Dept., AT&T Bell Labs, Murray Hill, NJ, 07974, [1]Dept. Biol. Sci., Univ. Maryland Baltimore County, Catonsville, MD 21228, and [2]Dept. Biophysics, East Tennessee State Univ., Johnson City, TN 37614.

The procerebrum (PC) of the terrestrial slug, *Limax maximus*, is a region of densely interconnected local olfactory interneurons within which the olfactory interneurons interconnect with (1) input fibers from the noses, (2) intrinsic modulatory fibers containing SCP_B or FMRFamide and (3) extrinsic modulatory fibers containing serotonin or dopamine.

To search for additional connections between the PC and other areas of the cerebral ganglion, localized injections of HRP were made into the cellular and synaptic zones of the PC. Fiber tracts into the olfactory nerve were seen, as were isolated single fibers connecting with the metacerebral lobe of the cerebral ganglion.

Material for fine structural analysis was fixed in glutaraldehyde and paraformaldehyde, postfixed in osmium tetroxide and stained in uranyl acetate and lead citrate. The cell mass consists of groups of very closely associated somata surrounded by bundles of axons. Within the synaptic interconnect zone fibers vary widely in size and orientation, containing both clear and electron-dense vesicles.

Dissociated PC neurons in culture are excited by serotonin and dopamine, while SCP_B and FMRFamide have little or no direct effect. Responses were assayed by whole-cell loose-patch recordings with pressure pulses of transmitter applied just upstream of the neuron being recorded.

NEURAL PLASTICITY IN ADULT ANIMALS: CEREBRAL CORTEX

338.1

RECOVERY OF FUNCTION AFTER CORTICAL DAMAGE: EFFECTS OF THE ANTICONVULSANT DRUG MK-801. T.M. Barth, M.L. Grant*, T. Schallert and S. D. Iversen*. Dept. of Psychology and Institue for Neurological Sciences, Univ. Texas, Austin, 78712

Previous research has shown that GABAergic anticonvulsant drugs retard or block recovery from sensorimotor asymmetries following unilateral cortical lesions (Watson and Kennard, 1945; Brailowsky et al., 1986; Schallert et al., 1986; Hernandez et al., 1988). Whether the detrimental effects of these drugs are due to the enhancement of GABAergic function or to their anticonvulsant properties has not yet been determined. In the present experiment we investigated the effects of MK-801, an anticonvulsant agent that is a noncompetitive antagonist at the N-methyl-D-aspartate (NMDA) receptor (Clineschmidt et al., 1982; Kemp et al., 1986; Wong et al., 1986), on recovery of behavioral function. Rats sustained unilateral electrolytic lesions in either the frontal medial (FMC) or sensorimotor cortex (SMC) and received either MK-801 (1 mg/kg) or saline injection beginning 12-16 hrs after surgery. Subsequent injections were given on days 2, 4 and 6. Behavioral tests measured sensorimotor asymmetries (i.e. a bilateral-tactile stimulation test) and forelimb placing. Unlike other anticonvulsant agents, MK-801 did not retard recovery. Indeed, results from rats sustaining FMC lesions suggested that MK-801 facilitated recovery from sensorimotor asymmetry ($p < .05$ for group; days; and the group X days interaction). A similar trend was found in rats with SMC lesions. An independent analysis of activity indicated that unlike GABAergic drugs (i.e. diazepam), MK-801 increased the percentage of time that the rats spent moving. These results are consistent with the view that the retardation of recovery observed after some anticonvulsant drugs may be due to the specific enhancement of GABAergic function. Supported by NIH grant NS-23964 awarded to T. Schallert

338.2

LC LESION ALTERS RAT SI CORTICAL PLASTICITY DUE TO ASSOCIATIVE TRAINING. C.L. Hand, R.L. Craik, and B.E. Levin. Univ. of Pa. Sch. of Vet. Med., Phila., PA 19104; Neurol. Svc., VA Med. Ctr., East Orange, NJ 17019.

Previous studies in neonatal & adult rat showed that a change in stimulus context--from a passive stroking (PS) to an associatively-paired training paradigm (AP; stroking + sugar water)--of a single vibrissa (C3) resulted in a metabolic shift in the C3 cortical barrel from a **decrease** of 5% to an **increase** of 4% from control values. Since other data suggest a role for norepinephrine (NE) in cortical plasticity, adult rats were subjected to a unilateral locus coeruleus (LC) lesion (6-hydroxydopamine) and assigned to one of 3 groups: controls (no training), PS bilaterally-trained C3 or AP bilaterally-trained C3. After 60 days training, effects were tested with 2DG, and cortical NE depletions were assayed (>60% depletion on lesioned side). Pilot data are remarkable for the differential changes in the size of the **areal representation.** While the PS rats (PS/lesioned & PS/nonlesioned sides) showed no significant areal changes, the AP rats (AP/lesioned & AP/nonlesioned sides) showed substantial increases in C3 barrel area compared to nonlesioned C3 control representations: AP/nonlesioned C3 barrel representations were 116% larger than controls. Introduction of the unilateral LC lesion (AP/lesioned) significantly ($p<0.05$) reduced the areal representation by 21% to an area only 90% larger than controls. These data suggest that NE plays a role in certain types of cortical plasticity.

338.3

PLASTICITY IN THE BARREL CORTEX OF ADULT MOUSE: EFFECTS OF PERIPHERAL DEPRIVATION ON THE FUNCTIONAL MAP; AN ELECTROPHYSIOLOGICAL RECORDING STUDY.

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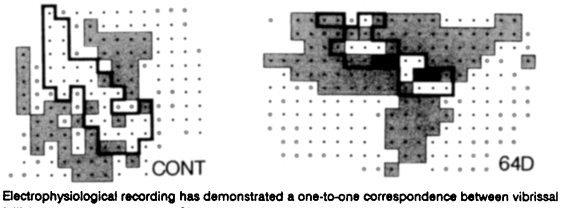

Electrophysiological recording has demonstrated a one-to-one correspondence between vibrissal follicles and barrels in mouse SI cortex. We here report on a mapping study using carbon fiber electrodes lowered into the barrel cortex under Nembutal anaesthesia. Penetrations were at 100 µm intervals. Posterior follicles of the C-row were denervated (Melzer et al., this Vol.). Maps of the barrel cortex contralateral to the lesion were made at 4 (n=1), 16 (n=4), 32 (n=2) and 64 (n=2) days after the lesion (*p.l.*), as well as from four control animals. After recording, whiskerpads and brains were processed for histology. At 16 and 32 days *p.l.* two types of maps could be distinguished: a) the C-zone was silent, i.e. no responses to whisker displacement could be elicited; b) the representations of whiskers of row B and D were enlarged, as were the receptive fields at individual penetrations; moreover, responses of affected follicles were obtained. The map of the 4 days *p.l.* mouse was of type a; those of the 64 days *p.l.* mice, of type b (see Fig; grey zones represent follicles of row B, lower left, and D; heavy line demarcates representation of follicles of row C). Recordings from affected follicles could be correlated with their reinnervation. Is the difference between type a and b depending on peripheral events, or does it reflect two modes of central adaptation to peripheral injury? Support: Swiss NSF grant 3.158.

338.4

BEHAVIORAL CONSEQUENCES OF FUNCTIONAL PLASTICITY IN RAT SI CORTEX PRODUCED BY CHRONIC SUBTOTAL VIBRISSAE DEAFFERENTATION. R.L.Craik, K.Gallo*, L.Lohwasser*, J.Lenich*, W.J. Carr*, C.L.Hand, and P.J.Hand. Beaver College, Glenside,PA 19038 and Sch. of Vet. Med., Univ. of PA., Phila,PA 19104.

Previous (^{14}C)2-deoxyglucose (2DG) studies revealed an enlarged and diffuse pattern of labeling of C3 representation in contralateral SI 90 days after neonatal follicle ablation sparing one vibrissa (C3). In a subsequent study rats kept the spared, enriched C3 vibrissa in contact with the walls of a cylindrical open field but preference dissipated with experience. Since the behavior extinguished in the open-field, this study examined behavioral changes in a more difficult task. On PND 1-3, 11 rats had follicle ablation unilaterally sparing C3; 8 animals were controls. On PND 60, rats were tested in a darkened cylindrical pen with a circular runway (5 cm wide) located against the inner wall of the cylinder 45 cm above the floor. Testing occurred 4 min per day for 5 days. It was predicted that animals with a right spared C3 would keep the vibrissa in contact with the wall traveling counterclockwise (CCW); left spared C3 animals would travel CW. (^{14}C)2DG experiments followed behavioral testing. The side of the C3 sparing affected behavior ($p<.05$) which did not extinguish; 9 of 11 spared C3 rats preferred the predicted mode unlike the control animals. The behavioral results correlate with the results of the 2DG tests suggesting that the elevated maze detects somatosensory behavioral changes and functional reorganization.

338.5

THE ROLE OF GABA IN RECOVERY OF FUNCTION AFTER CORTICAL LESIONS. T.D. Hernandez, T.A. Jones* and T. Schallert. Dept. Psychology and Institute for Neurological Sciences, Univ. of Texas, Austin 78712.

Recovery from sensorimotor asymmetries caused by unilateral damage to the cortex is disrupted by chronic administration of GABA or GABAergic drugs (Watson and Kennard, 1945; Hernandez et al., 1985, 1987, 1988; Brailowsky et al., 1986a,b, 1987; Schallert et al., 1986). Diazepam chronically delays recovery from an otherwise short-term somatosensory asymmetry caused by unilateral anteromedial cortex (AMC) lesions in rats (Schallert et al.,1986; Hernandez et al., 1987, 1988). One possible site of action for these agents is adjacent cortical tissue. Indeed, Brailowsky et al.(1986a) found that the hemiplegic syndrome produced by cannula-induced motor cortex damage is exacerbated and prolonged by chronic infusions of GABA into the surrounding motor cortex. In the present study, following unilateral AMC lesions, the GABA agonist, muscimol, was infused into the adjacent sensorimotor cortex (SMC) or, as a control, into the more remote visual cortex (VC) of the ipsilateral hemisphere. Infusions into the SMC, but not VC, retarded recovery. These data suggest that diazepam might interfere with recovery from AMC lesion-induced somatosensory asymmetry, in part, via GABA-enhancing action within the nearby SMC. In addition, we found that diazepam did not disrupt recovery from SMC lesions, which typically yield a more long-term behavioral asymmetry. These and previously reported data indicate that the vulnerability of the recovery process to GABAergic manipulations may vary depending on anatomical, neurochemical and behavioral factors.

Supported by grants from The Ford Foundation and NIH (NS-23964).

338.6

PLASTICITY IN THE BARREL CORTEX OF ADULT MOUSE: EFFECTS OF PERIPHERAL DEPRIVATION ON THE FUNCTIONAL MAP; A DEOXY-GLUCOSE STUDY.

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In the adult mouse whisker-to-barrel pathway, the deoxyglucose (DG) method has shown that the deflection of whiskers evokes an increase in neuronal metabolism confined to the corresponding barrels. Is this *metabolic map* in the CNS modified after denervation of whisker follicles in the periphery? In 28 adult albino mice, the branch of the infraorbital nerve which innervates the vibrissae of row C on the left muzzle was ligated and divided just distal to the ligature. At 1, 4, 8, 16, 32, 64, and 160 days post lesionem (*p.l.*), the animals were injected with [^{14}C]2-DG, and left vibrissae B1-3 & D1-3 were stimulated; all other whiskers were clipped (Melzer, P., et al., Brain Res., 348:229, 1985). Three non-injured mice underwent the same stimulation experiment. Autoradiography revealed that already one day *p.l.*, contralateral to injury and stimulation, DG uptake in barrels C1-3 was higher than normal. The increase occurred first at the borders to rows B & D and was continuous with the area of DG uptake in barrels B1-3 & D1-3, respectively. On average, the area over row B was the larger. In harmony with the increase in DG uptake in barrels C1-3, DG uptake rose in the representation of row C in the brainstem subnuclei caudalis & interpolaris ipsilateral to stimulation. In the ipsilateral barrelfield, DG uptake never differed from normal. At 4 days *p.l.*, myelinated fibers had disappeared from follicles C1-3. They re-appeared at 64 days *p.l.* In spite of this re-innervation, the increase in stimulus-evoked DG uptake over the representations of the (non-stimulated!) vibrissae C1-3 persisted and was highest at 160 days *p.l.* We attribute the observed changes to an enlargement of the metabolic representation of the whiskers neighboring those whose sensory innervation had been severed. The mechanisms underlying the alteration of the central *metabolic map* as well as the persistence of the changes upon peripheral re-innervation remain to be revealed. Support: Swiss NSF grant 3.158.

338.7

Lesions of Basal Forebrain alter Stimulus-evoked Metabolic Activity in Mouse Somatosensory Cortex. W. Ma, C.F. Hohmann, J.T. Coyle, & S.L. Juliano, USUHS, Bethesda, MD and Johns Hopkins University, Baltimore, MD.

Although the cholinergic innervation of the cerebral cortex has been reported to selectively increase the cortical response to specific stimuli, there has been little demonstration of its effect on activity patterns in sensory systems. To address this issue, we initiated experiments to study the effect of neurotoxic lesions of the basal forebrain (NBM) on stimulus-evoked metabolic activity in the barrel field of mouse somatosensory cortex. One month following a unilateral ibotenic acid lesion of the NBM, ^{14}C-2-deoxyglucose (2DG) was injected IP into 16 Balb-C mice. During the 2DG experiment, each mouse received a bilateral stimulation to the C-3 vibrissa. The lesion sites were later identified using acetylcholinesterase and cytochrome oxidase (CO) histochemistry on sections adjacent to the 2DG sections and using Nissl stains obtained from the sections which produced the 2DG autoradiograph. Comparison of the column-like label evoked after stimulation of a single whisker on each side revealed that the activity on the lesioned side was significantly reduced from that found in the normal hemisphere. On the normal side, the activated barrels averaged 638um in tangential width, were 51% above background in density, and extended from lamina II-V. On the lesioned side, the activated barrels were 470um in width, were 40% above background in density and extended from upper layer II-IV. Cortical 2DG activity outside the stimulated barrel area was the same on each side. CO activity observed on adjacent sections revealed no differences in the staining pattern for this mitochondrial enzyme in the normal and lesioned hemispheres. These experiments suggest that acetylcholine plays a significant role in the processing of somatosensory information and that its absence diminishes the stimulus-evoked activity, while the underlying metabolic activity is either not affected or recovers over time. Supported by RO7064.

338.8

Manipulation of Cortical Cholinergic Innervation Alters Stimulus-evoked Metabolic Activity in Cat Somatosensory Cortex. S.L. Juliano, W. Ma, M. Bear, and D. Eslin . USUHS, Bethesda, MD, and Brown U, Providence, RI.

Acetylcholine has (ACh) recently been reevaluated regarding its role in cerebral cortical activity. It now seems clear that this neurotransmitter is involved with increasing the specificity of cortical response. Nevertheless, little is known about the participation of ACh in processing sensory stimuli. To address this issue, we used 2 methods to manipulate the supply of ACh in the somatosensory cortex (SSC) of cats: (i) topical application of the cholinergic antagonist, atropine, (n=5) and (ii) unilateral neurotoxic lesions (using NMDA) of the basal forebrain (n=3). Twenty minutes after application of atropine and 1 week after the NMDA lesion, each animal underwent a 2-deoxyglucose experiment while the animal received identical somatic stimuli to each forepaw. The results obtained from the topical applications of atropine indicated that high doses (100u M) almost eliminated the stimulus-evoked activity in the SSC, while lower doses (40u M) reduced its density and distribution. The metabolic patterns of cortical activity were most often in the form of patches on individual sections and were reconstructed in 2 dimensions throughout the SSC. The reconstructed distributions formed strips which extended in the rostro-caudal plane & revealed that the patterns on the treated sides were similar in their overall distribution but differed in their dimension and density. For example, after a unilateral topical application of 40u M atropine, the strip width averaged 680um, while in the untreated hemisphere the strips measured 777um in width. The density of the metabolic label was 59% above background on the treated side and 79% above background on the normal side. The NMDA lesions caused similar reductions in the distribution and density of metabolic label. These changes indicate that ACh plays a significant role in the processing of sensory information and the formation of somatosensory cortical columns.

338.9

PLASTICITY IN PRIMARY SOMATOSENSORY CORTEX AFTER DIGITAL NERVE SECTION AND REGENERATION IN ADULT OWL MONKEYS. T. ALLARD, S.A. CLARK*, K.A. GRAJSKI, and M.M. MERZENICH. Coleman Lab., Depts. of Otolaryngology and Physiology, UCSF Med.Ctr., San Francisco, CA 94143; (SAC) Dept. of Plastic Surgery, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

In digital nerve section and regeneration, regenerating fibers reinnervate different peripheral sites disrupting the original topographical relationship between peripheral inputs and their central targets. The response of cortical neurons to this disruption and reassignment of peripheral inputs was measured in area 3b of adult owl monkeys. In the first study, single digital nerves were sectioned and reconnected near the metacarpal-phalangeal joint and allowed to regenerate into their original skin fields on the finger. In the second study, single digital nerves from one finger were sectioned and cross-connected to digital nerves of an adjacent finger. Highly detailed cortical maps were reconstructed from multiunit receptive fields (RFs) defined at many microelectrode penetration sites across the cortex 6 months and over a year following nerve reconnection. After final cortical mapping sessions, the skin fields of regenerating and adjacent digital nerves were mapped with the evoked potential technique. In some cases, RFs were defined in the ventral posterior lateral nucleus of the thalamus.

Both central mapping and peripheral evoked potential studies showed that denervated skin fields were completely reinnervated by cutaneous inputs. Peripheral evoked potentials showed inputs from the target skin fields were conveyed solely through fibers of the regenerating digital nerves with no observable reinnervation from adjacent nerves. In the uncrossed nerve reconnection study, the cortical representation of the regenerating nerve and adjacent skin showed normal features of map orientation and internal topography: there was a normal abrupt discontinuity in the cortical map of adjacent digits and a progressively-shifted overlap of receptive fields from the normally innervated half of the finger to the reinnervated half of the finger within the representation of a single digit. Some cortical RFs from the reinnervated skin surface were unusually large. In the crossed-nerve study, regenerating nerves were represented in their original relative position in the cortical map and in a new cortical zone near their newly-adjacent peripheral inputs.

These studies show that central RFs in adult somatosensory cortex are dynamically created and maintain an orderly internal topography following the resorting of peripheral inputs in regenerating digital nerves.

Supported by NIH grant NS-10414, the Coleman Fund, and HRI.

338.10

INNERVATED NEOCORTICAL GRAFTS CAN BE ACTIVATED BY ADULT HOST SOMATOSENSORY INPUTS. S.M. Lee and F.F. Ebner, Center for Neural Science, Brown University, Providence, RI 02912

Grafts of embryonic neocortex implanted in the mature barrel field become innervated by VB nucleus fibers when the appropriate peripheral nerve is transected 2 days prior to grafting (Erzurumlu and Ebner, JCN, 1988). The present experiments were carried out to determine whether innervated graft neurons show physiological responses to peripheral stimulation after the transected nerve regenerates.

The infraorbital (IO) nerve was transected unilaterally in adult male Long Evans rats (n=5) under Nembutal anesthesia. Two days later pieces of E19-20 parietal cortex were implanted in barrel field cortex contralateral to the sectioned IO nerve. The control animals (n=4) received similar transplants, without prior sensory nerve transection. From 40 to 60 days after grafting the animals were anesthetized with urethane and the barrel field cortex containing the graft was re-exposed for physiological analysis. Single unit responses to electrical stimulation of the regenerated IO nerve (0.5 µA, 200 µsec) and/or glutamate iontophoresis in cortex (0.5 M, pH 7.0, 23 nA) were recorded on-line with a 3-barrelled carbon fiber electrode (1-5 MΩ @1 kHz) and stored on a computer. Each recording site was marked with an electrolytic microlesion (1 mA, 30 sec) that could be localized following cytochrome oxidase histochemistry.

Of 72 neurons isolated by extracellular single unit recording, 45 were localized histologically within the transplant. Approximately 20% of the graft neurons analyzed were responsive at short (7-14 msec) latency to electrical stimulation of the regenerated IO nerve. Following iontophoretic application of glutamate an additional 13% could be activated by sensory nerve stimulation. In contrast, all control graft neurons isolated by glutamate application showed almost no spontaneous activity and were unresponsive to IO nerve stimulation These findings indicate that the sensory fiber ingrowth induced by peripheral nerve damage leads to functional innervation of grafted neurons.

(supported by NIH grant NS 13031 and the Mathers Foundation)

338.11

ALTERATIONS IN DENDRITIC EXTENT OF PYRAMIDAL CELLS IN ADULT MOUSE CORTEX: A QUANTITATIVE GOLGI STUDY OF THREE AGE GROUPS. Ronald F. Mervis, Monika Bedo-Wierdl*, and Robert Dvorak*. Div. of Neuropathology, The Ohio State Univ. Med. Ctr., Columbus, Ohio 43210

In order to establish baseline lifespan parameters of neuronal morphology and assess neuroplasticity in the brains of adult rodents, we evaluated the extent of dendritic branching in cortical neurons in early- through middle-adulthood in male C57Bl/6J mice. Three ages were studied: 3, 8, and 13 months-old. Using Rapid Golgi preparations, the dendritic domain of the basilar tree of randomly selected layer V pyramidal cells from fronto-parietal cortex were quantified by Sholl analysis. Results showed that the amount of dendritic material in the 8 month-old mice was significantly reduced compared to that of the 3 month-old mice: there was a 34% decrease in dendritic material. There was no additional reduction in dendritic extent of pyramidal cells between the 8 month-old mice and the 13 month-old mice. These findings suggest that in this rodent the transition from early to middle adulthood is associated with a extensive reorganization of dendritic arbor as shown by the reduction of dendritic material. However, in the 8 to 13 month-old range the dendritic field was shown to be stabile. (Supported by Central Soya, Dept. of Pathology, and the Office of Geriatrics and Gerontology).

338.12

FLUORESCENT STAINING OF THE CEREBRAL CORTEX IN LIVING MICE. A-S. LaMantia, S.L. Pomeroy, and D. Purves, Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110

We have evaluated the ability of several vital dyes to stain the superficial layer of the cerebral cortex in living mice. Young adult male mice (CF1 strain, 25-30g) were anesthetized and placed on the stage of a modified compound microscope (Purves et al., J. Neurosci 6: 1051, 1986). Following a midline incision and 3 mm occipital craniotomy, the dura was reflected to expose the cortical surface. To enhance penetration of dyes through the pia/arachnoid, 0.01% saponin in lactated Ringer's was superfused over the cortex, followed by 10 μM dye solutions, each for 3 min. Electron microscopic analysis of detergent treated, fluorescent stained cortex fixed immediately after imaging revealed only moderate disruption of cortical cytology -- most notably, swelling of dendritic profiles in layer 1.

Of 13 nuclear dyes tested, bis-benzamide (Hoechst 33342) and DAPI (Sigma) gave the brightest and most consistent staining of nuclei within the pia/arachnoid; there was little nuclear staining beneath this layer. Of 23 cytoplasmic dyes examined, three styryl pyridinium dyes (4-Di-1-ASP, 4-Di-2-ASP, and 4-Di-5-ASP; Mol. Probes Inc.) were most effective. Small bipolar cells with fine, beaded processes extending at least 200 μm were stained completely; these cells were usually directly apposed to blood vessels. Each dye also revealed a granular pattern deep to the pia which probably represents layer I neuropil. Corollary observations of styryl pyridinium staining in other regions of the CNS support this conclusion. Thus in the olfactory bulb staining is confined to the glomerulae, which contain olfactory afferent terminals, and mitral cell and peri-glomerular cell dendrites. These in vivo observations are generally consistent with standard morphological descriptions, and suggest that vital fluorescent staining and imaging may be useful techniques for monitoring the anatomy of the central nervous system over time. (NIH NS18629 and 11699.)

338.13

ONTOGENY OF NEURONAL DISCHARGE PATTERNS IN THE OCCIPITAL CORTEX OF CHLORAL-HYDRATE ANAESTHETIZED RATS. M.A. Corner and M. Mirmiran*. Netherlands Institute for Brain Research, 1105 AZ Amsterdam.

Male Wistar rats varying in postnatal age from 2 to 12 weeks were studied under chloral hydrate anaesthesia, using glass microelectrodes to record spontaneous firing in single neurons at different depths within the occipital cortex. Computerized quantification of the spike trains revealed a wide variety of complex patterns which were unrelated either to cortical depth or to fluctuations in the local EEG. Nevertheless, significant and persistent changes of interneuronal profiles occurred in an orderly fashion for different parameters at different ages:
i) at 3-4 weeks, the modal values of the interval histograms decreased from ca. 25 msec (group median) to 4-5 msec; ii) at 4-5 weeks, the long-lasting clusters of relatively intense spike activity which are characteristic for immature animals became shorter by about two-thirds; iii) between 5 and 8 weeks, slow (longer than 2-3 min) fluctuations in the mean firing rate became half as small, on the whole; iv) between 8 and 12 weeks, prominent high-frequency rhythmic components (varying from 2 to 15 cps) appeared in the spontaneous firing of many neurons.

338.14

NEONATAL 6-OHDA AND ENRICHED REARING CONDITIONS: EFFECTS ON REGIONAL BRAIN ^{14}C-2-DEOXYGLUCOSE UPTAKE ARE CRITICALLY DEPENDENT ON AROUSAL AT TEST TIME. J.N. Nobrega, M.J. Saari, J. Armstrong* & T. Reed*. Clarke Institute of Psychiatry, Toronto, Ont., M5T 1R8, Canada, and Nipissing University College, North Bay, Ont., P1B 8L7, Canada.

A preliminary investigation had suggested that relatively few brain areas show changes in ^{14}C-2-deoxyglucose (2DG) uptake as a result of norepinephrine (NE)-depleting neonatal 6-OHDA treatment and/or rearing conditions. The present study examined the possibility that 2DG profiles in these animals might vary as a function of arousal state at test time. Forty-eight newborn male Wistar rats received s.c. injections of 6-OHDA or vehicle and were reared in "isolated" or "enriched" conditions (Neurosc. Abstr., 1987, 13, 406). ^{14}C-2DG procedures were performed when the rats were three months old. Rats received the 2DG injection (100 μCi/kg i.v.) and either stayed in the home cage, or were placed in a novel environment, an open field in a separate room, for 45 min before sacrifice. Overlaying of stained sections and autoradiographic images was used to quantitate label uptake (nCi/g tissue) in 97 brain areas. Analyses of variance revealed significant Open Field X 6-OHDA interaction effects in locus coeruleus, posterior hypothalamus, and three thalamic nuclei. Twenty other areas showed significant Open Field X Enrichment interaction effects. An Enrichment main effect was seen in the anterior pretectal area, somatosensory cortex, and three thalamic nuclei. These results suggest that the effects of early NE depletion and rearing conditions on the metabolic activity of a number of brain areas may only become apparent in the presence of arousing stimulation.

SEROTONIN, HISTAMINE AND OTHER BIOGENIC AMINES VI

339.1

5-HYDROXYTRYPTAMINE ACTS AT 5-HT$_2$ RECEPTORS TO REDUCE G_K IN RAT NUCLEUS ACCUMBEN NEURONS. N. Uchimura* and R.A. North, Vollum Institute, Oregon Health Sciences Univ., Portland, OR 97201.

Intracellular recording were made from rat nucleus accumbens neurones in brain slices. 5-hydroxytryptamine (5HT, 1-100 μM) produced depolarization associated with an increase in the input resistance. Application of 5-HT to neurons voltage-clamped caused an inward current and a decrease in the slope conductance. The current caused by 5-HT was inward at the resting potential (typically about -80 mV) and reversed to outward at the potassium equilibrium potential. This reversal potential was linearly related to the logarithm of the extracellular potassium concentration. The depolarization caused by 5-HT persisted in tetrodotoxin (1 μM). It was not abolished by a solution that contained lower levels of calcium (0.24 mM), higher levels of magnesium (5 mM), and cobalt (2 mM). The 5-HT depolarization was competitively antagonized by ketanserin and mianserin with apparent dissociation equilibrium constants of about 3 nM and 45 nM, respectively. The depolarization was not mimicked or blocked by a number of agonists and antagonists selective for the 5-HT$_1$ and 5-HT$_3$ receptor. These results show that neurons of the rat nucleus accumbens are depolarized by 5-HT acting at 5-HT$_2$ receptors to reduce to conductance of the membrane to potassium ions.

339.2

CHARACTERIZATION OF 5-HT$_2$ RECEPTORS IN THE RAT FRONTAL CORTEX: ELECTROPHYSIOLOGICAL STUDIES. E. Edwards, K. Harkins*, L.H. Jiang, R.J. Kasser, C.R. Ashby, Jr. and R.Y. Wang, Dept. of Psychiatry & Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794.

There is strong evidence for 5-HT$_2$ involvement in the mechanism of action of hallucinogenic agents. Receptor binding autoradiography have revealed a high concentration of 5-HT$_2$ binding sites in the rat frontal cortex (FC). The aim of the present study was to characterize 5-HT$_2$ receptors using the techniques of single cell recording and microiontophoresis (ionto). Ionto-5-HT, 5-HT$_{1a}$ receptor agonist 8-hydroxy-2-[dipropyl-amino]-tetralin (8-OH-DPAT), 5-HT$_{1b}$ receptor agonist m-chlorophenylpiperazine (mCPP) and 5-HT$_2$ receptor agonist [1-4-iodo-2,5-dimethoxyphenyl]2-aminopropane (DOI) on FC cells all suppressed the neuronal activities in the FC. The 5-HT$_2$ receptor antagonist ketanserin partially blocked the depressant effect of DOI but not that induced by other 5-HT agonists. In fact, ketanserin potentiated the effect produced by 5-HT, 8-OH-DPAT and mCPP. The blockade of DOI by ketanserin cannot be due to its anti-α_1-adrenoceptor effect because α_1-adrenoceptor antagonist prazosin did block the action of DOI. To further characterize 5-HT$_2$ receptor subtypes in the FC requries more specific 5-HT$_2$ agonists and antagonists. (Supported by USPHS grants MH-41440, MH-41696 and MH-00378 to R.Y.W and BNS-8614098 and MH-44048 to E.E).

339.3

5-HT_2 RECEPTORS IN RAT PREFRONTAL CORTEX. R.C. Araneda* and R. Andrade (SPON: K. Shibata). Dept. of Pharmacology, St. Louis Univ. School of Med., St. Louis, MO 63104.

While the pharmacology, distribution and biochemistry of central 5-HT_2 receptors have been extensively investigated, little is still known about the physiological responses they mediate. Therefore we have used intracellular recordings in in vitro rat cortical slices to examine the effects of 5-HT on the prefrontal cortex, an area highly enriched in these receptors.

Bath administration of serotonin (.1 µM - 30 µM) elicited three distinct spiperone and ketanserin sensitive responses. These included a small, subthreshold depolarization associated with a conductance decrease, a reduction in the slow afterhyperpolarization which follows a burst of spikes, and a marked decrease in spike frequency accommodation. In addition, when 5-HT_{1a} and 5-HT_2 receptors coexisted on the same cell, activation of the 5-HT_2 receptors reduced or blocked the ability of the 5-HT_{1a} receptors to hyperpolarize these cells.

Thus, activation of 5-HT_2 receptors in this region elicits a set of distinct actions which interact to produce an increase in excitability to incoming stimuli while blocking the inhibitory actions of 5-HT.

Supported by the Pharmaceutical Manufacturers Association Foundation.

339.4

EFFECTS OF KETANSERIN PRETREATMENT ON MDMA-INDUCED SEROTONIN (5-HT) DEPLETION IN THE RAT. J.F. Nash and H.Y. Meltzer, Department of Psychiatry, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106

Previous studies conducted in our laboratory have established that +3,4-methylenedioxymethamphetamine (MDMA) stimulates the secretion of corticosterone in a dose- and time-dependent manner, and that ketanserin pretreatment prevents this effect of MDMA. MDMA has been reported to deplete brain concentrations of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in a biphasic manner. The present study was undertaken to determine the effect of ketanserin pretreatment on acute (3 hr) and chronic (7 day) MDMA-induced 5-HT and 5-HIAA depletion. A single injection of MDMA (20 mg/kg, s.c.) significantly reduced the concentration of 5-HT and 5-HIAA in the following brain areas: frontal cortex > hippocampus=striatum > hypothalamus at 3 hr and 7 days. Treatment of rats with ketanserin (3 mg/kg, i.p) 1 hr prior to MDMA administration significantly attenuated MDMA-induced depletion of 5-HT and 5-HIAA at both time points. Ketanserin prevented MDMA-induced depletion most significantly in the striatum followed by the hippocampus and frontal cortex. MDMA-induced depletion of 5-HT and 5-HIAA in the hypothalamus was unaffected by ketanserin. These data suggest that reducing the acute MDMA-induced depletion of 5-HT and 5-HIAA by ketanserin pretreatment attenuates the long-term depletion. We propose that overstimulation of 5-HT_2 receptor mechanisms may, in part, account for the long-term depletion of 5-HT following a single administration of MDMA.

339.5

ARACHIDONIC ACID (AA) METABOLISM REGULATES GENERATION AND DESENSITIZATION (DEZ) OF A 5-HT_2 RECEPTOR- MEDIATED RESPONSE. B.Dalton*, J.Kaplan*, S.Maayani and R.R.Ben-Harari* (SPON: J.Goldfarb). Depts. of Pharmacology and Anesthesiology, Mt.Sinai Sch. Med., CUNY, N.Y., N.Y.10029.

Intracellular mediators, such as eicosanoids, modulate DEZ of a variety of receptor systems in smooth muscle. The response to 5-HT on guinea-pig trachea undergoes rapid DEZ and is 5-HT_2 receptor-mediated. The effect of inhibitors of eicosanoid metabolism on this response and DEZ was studied by novel kinetic procedures utilizing on-line computer digitization.

Drug(concentration; site of action)	Peak tension	DEZ rate
Indomethacin (1uM; cyclooxygenase)	↑	=
Nordihydro-guaiaretic acid (NDGA) (30uM; lipoxygenase and cytP450 oxygenase)	=	↑
Clotrimazole (Clo) (1uM; cyt P450 oxygenase)	↑	↓
Clo + NDGA	↑	↑
Arachidonic acid (AA) (10uM)	=	↓
AA + INDO	↑	↑
AA + NDGA	=	↑

We propose that the AA cascade participates both in generation and in DEZ of 5-HT_2-mediated responses. (Supported by USPH GM-34852)

339.6

SEROTONINERGIC (5-HT_2) REGULATION OF PLASMA CORTICOSTERONE AND PROLACTIN. C.Cosi, H.S.Kim and P.L.Wood. Res. Dept., Pharmaceut. Div., CIBA-GEIGY, Summit, NJ 07901.

The effect of DOI [1-(2,5-dimethoxy-4-iodophenyl-)2-aminopropane], a 5-HT_2 agonist, on plasma corticosterone and prolactin (PRL) was investigated in rats. PRL and corticosterone were detected in the same sample by radioimmunoassays. DOI (0.2-0.5 mg/kg ip) increased corticosterone to 277% and 325% of control respectively, in a dose-dependent manner. PRL was increased to 367% of control by 0.5 mg/kg DOI. In a time course experiment, DOI (0.2 mg/kg) increased corticosterone to 565% and 292% of control at 30 and 45 min respectively. DOI increased PRL to 392% of control at 30 min. At 60 min after DOI, both corticosterone and PRL had returned to control levels. Pretreatment with cinanserin (10 mg/kg ip), a 5-HT_2 antagonist, completely suppressed the action of DOI and did not affect, by itself, corticosterone or PRL. These results indicate that 5-HT_2 receptors are involved in mediating serotonin increases of plasma corticosterone and PRL.

339.7

5-HT-3 BINDING SITES IDENTIFIED IN RAT BRAIN USING [^{3}H]-ICS-205-930 A.W. Schmidt, C.J. Siok and T.F. Seeger* Pfizer Central Research, Groton, CT 06340

Serotonin receptors have been classified into multiple subtypes designated 5-HT-1, 5-HT-2 and 5-HT-3. 5-HT-1 and 5-HT-2 receptors have been extensively studied in brain, while knowledge about 5-HT-3 receptors largely has been accumulated from studies on peripheral tissues. More recently, 5-HT-3 binding sites have been identified in rat brain using [^{3}H]-GR-65630. We now report and confirm the identification of 5-HT-3 binding sites in rat brain using the tritiated form of the specific 5-HT-3 receptor antagonist ICS-205-930. Saturation experiments utilizing homogenates of rat frontal and entorhinal cortices show that binding occurs with high affinity (K_d = 1.30 nM) and a binding capacity of 29.2 fmol/mg protein. Specific 5-HT-3 antagonists inhibit [^{3}H]-ICS-205-930 with nanomolar affinity (ICS-205-930 > GR-38032F > MDL-72222). The rank order of affinity agrees well with the relative potency of these compounds as antagonists of 5-HT induced bradycardia (Bezold-Jarisch reflex), a 5-HT-3 mediated event. Agonists inhibited [^{3}H]-ICS-205-930 binding with lower affinity than antagonists (5-HT > phenylbiguanide > 2-methyl-5-HT). Other serotonergic agents (8-OH-DPAT, methysergide, cinanserin) did not significantly inhibit binding at 1 uM. Preliminary autoradiographic studies reveal a heterogeneous distribution of radiolabel in the rat brain. Highest levels of binding were found in entorhinal and frontal cortices with moderate levels of binding in limbic areas (amygdala, hippocampus, n. accumbens/olf. tub).

339.8

5-HT_3 RECEPTORS IN THE RABBIT RETINA? W.J.Brunken & N.W.Daw Dept of Biology, Boston College Chestnut Hill, MA 02167 and Dept of Cell Biology, Washington Univ. St. Louis MO 63110

We have suggested previously that both 5-HT_1 & 5-HT_2 receptors have a role in signal processing in the rabbit retina (JNS 7:1054-1065). In preliminary experiments we have employed MDL 72222 and ICS 205-903, serotonin antagonists selective for 5-HT_3 receptors in a superfused isolated eyecup preparation to test if 5-HT_3 receptors affect the visual responses of the ganglion cell, the output neuron of the retina.

These drugs had reversible effects on the visual responses of both ON and OFF center brisk cells. For all cells tested, the ON-excitation was reduced and in some cells the OFF-excitation was increased. The spontaneous activity of all classes of cell was also decreased. These effects were reversed in 10 to 30 minutes after the drugs were washed out of the perfusion bath.

The results obtained with these agents are similar in all respects as those obtained with agents selective for 5-HT_2 receptors. These results may imply either that 5-HT_3 receptors are present in the mammalian retina or that the reputed 5-HT_3 agents have actions at central 5-HT_2 receptors. Further experimentation will be necessary to confirm a functional role for 5-HT_3 receptors in the retina. (Sponsored by EY 06776 WJB and EY 00053 NWD)

339.9

SEROTONIN-3 RECEPTOR MEDIATED SPINAL ANTI-NOCICEPTION. S.R.Glaum and E.G.Anderson, Dept. of Pharmacology, U of I/Chicago. Chicago IL 60680

We recently reported that the potent and selective $5HT_3$ receptor antagonists ICS 205-930 and MDL 72222 inhibit specific ^{3}H-Serotonin binding to purified rat dorsal spinal cord synaptosomal membranes (Eur.J.Pharm.,submitted). Intrathecal (IT) administration of 5HT is known to produce significant antinociception as measured by increased tail-flick latency (TFL). We now report that the selective $5HT_3$ receptor agonist 2-methyl-5HT mimics the antinociceptive action of 5HT. Additionally, the selective $5HT_3$ receptor antagonist ICS 205-930 inhibits 5HT induced antinociception.

50,100 or 200µg IT infusions of 5HT or 2-methyl-5HT produced dose-dependent increases in TFL ($p<0.01$) at 5, 15 and 25 min post-injection. Treatment with 0.1-10µg ICS 205-930 alone produced no significant change in TFL. However, pretreatment with 0.1-1.0µg ICS 205-930 5 min prior to 200µg 5HT completely blocked anti-nociception. Furthermore, administration of 10µg ICS 205-930 prior to 200µg 5HT produced a significant decrease in TFL (hyperalgesia). These findings strongly implicate $5HT_3$ receptors in mediating spinal analgesia. PHS NS 17834-04.

339.10

5-HYDROXYTRYPTAMINE INFLUENCES RELEASE OF DOPAMINE AND ITS METABOLITES FROM RAT STRIATAL SLICES. P. Blandina* and J.P. Green. Department of Pharmacology, Mount Sinai School of Medicine, City University of New York, New York, NY 10029.

Serotonergic fibers originating from the dorsal raphe nucleus project into the striatum but the function of 5-hydroxytyptamine (5-HT) in striatum is still unclear since the neurotransmitter has been reported to have both excitatory and inhibitory effects (Chesselet M.F., Neurosci. 12, 347, 1984). The role of 5-HT in the release of endogenous dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) from superfused striatal slices was assessed. Eight week old, male Sprague-Dawley rats were decapitated, and striatal slices (400 µm thick) were superfused with artificial cerebrospinal fluid (aCSF) containing nomifensine (10 µM) to block DA reuptake. Measurements were made by high performance liquid chromatography with electrochemical detection. Spontaneous release of DA from the slices averaged 1.9 pmol/mg protein/3 min. The release of DOPAC and HVA averaged 3.5 and 1.1 pmol/mg protein/3min, respectively. Superfusion with 5-HT (10µM) resulted in a 3-fold elevation of DA release with no significant changes in the release of DOPAC and HVA. The 5-HT agonists 5-carboxyamidotryptamine (1 µM) and 2,5-dimethoxyphenylisopropylamine (10 µM) were ineffective in increasing the spontaneous release of DA, DOPAC or HVA. 2-Methyl-5-HT (10µM), an agonist of 5-HT selective for the 5-HT3 receptor, produced a 3-fold increase in spontaneous release of DA without altering the release of DOPAC or HVA. The 5-HT3 receptorshas been described in the peripheral nervous system where they appear to mediate the excitatory effects of 5-HT. These data suggest that the 5-HT3 receptor is present in brain as well. Supported by a grant (DA 01875) from the National Institute on Drug Abuse.

339.11

EXAMINATION OF THE EFFECTS OF THE 5-HT_3 RECEPTOR ANTAGONIST MDL-72222 ON PUNISHED RESPONDING IN PIGEONS. S.T. Ahlers and J.E. Barrett. Department of Psychiatry, Uniformed Services University of the Health Sciences and the Naval Medical Research Institute, Bethesda, MD 20814.

MDL-72222, a 5-HT_3 receptor antagonist, was examined for potential anxiolytic activity using a behavioral assay that, in general, has correlated well with clinically efficacious anxiolytics in humans. Pigeons were trained to key peck under a multiple fixed ratio (FR) 30-response schedule in which different components were correlated with a red or white keylight color; components alternated every 3 min. During one component, the 30th response also produced a brief electric shock which suppressed responding (punishment). MDL-72222 at doses from 0.001 to 3.0 mg/kg, i.m. administered immediately before the session produced slight increases in punished responding of some pigeons within a narrow dose range of 0.1-0.3 mg/kg. Unpunished response rates were unaffected across most of the dose response curve. Overall, the effects of MDL-72222 on punished responding were relatively weak compared to buspirone or chlordiazepoxide which have robust effects under this procedure and show potent anxiolytic activity in humans. Supported by PHS Grant DA-02873.

339.12

COMPARATIVE BEHAVIORAL EFFECTS OF SEROTONIN-3 ANTAGONISTS AND DOPAMINE ANTAGONISTS IN RODENTS. **A.E. Williams*, J.N. Wiley* and T.G. Heffner (SPON: D.A. Downs). Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.**

Based on reports of possible antipsychotic utility of serotonin-3 ($5HT_3$) antagonists, we examined the effects of the reported $5HT_3$ antagonists GR 38032F (GR), MDL 72222 (MDL), and ICS 205930 (ICS) in locomotor activity tests in rodents. In mice, MDL reduced spontaneous locomotion with an ED_{50} of 5 mg/kg IP and did not cause ataxia at active doses, a profile seen with dopamine antagonists. ICS reduced locomotion at higher doses (ED_{50} = 28 mg/kg IP) in mice and also did not cause ataxia. GR did not reduce locomotor activity in mice at doses of 0.001 - 3 mg/kg IP; higher doses were lethal. In rats, MDL reduced locomotor activity after oral dosing with an ED_{50} of 8 mg/kg; GR and ICS were inactive at doses up to 10 and 100 mg/kg PO, respectively. GR failed to reduce locomotion in rats after SC or IP dosing with up to 10 mg/kg. Neither acute nor BID dosing with GR for 3 successive days reduced d-amphetamine-induced hyperactivity in mice. In contrast, both MDL and ICS antagonized amphetamine stimulation in mice. These results indicate that while the profiles of MDL 72222 and ICS 205930 in these tests share common features with those of dopamine antagonists, this activity is not seen with the other $5HT_3$ antagonist, GR 38032F.

339.13

EFFECTS OF THE SEROTONIN-3 (5HT-3) ANTAGONIST, GR38032F, ON MIDBRAIN DOPAMINE (DA) NEURONS. C.L. Christoffersen, K.A. Serpa, and L.T. Meltzer. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

GR38032F (1,2,3,9-tetrahydro-9-methyl-3-[(2-methylimidazol-1-yl)methyl]-4H-carbazole-4-one, hydrochloride) is a potent and selective 5HT-3 receptor antagonist (Brittain et al., Br. J. Pharmac. 90: 87P, 1987) that has been shown to decrease mesolimbic DA hyperactivty in behavioral and biochemical experiments (Costall et al., Br. J. Pharmac. 92:881, 1987; Hagan et al., Eur. J. Pharmac. 138: 303, 1987). In the present experiments we have begun to evaluate the effects of GR38032F on the firing activity of midbrain DA neurons, recorded extracellularly in chloral hydrate anesthetized rats. IV administration of GR38032F in cumulative doses up to 1 mg/kg had no effect on the baseline firing rate of DA neurons in either the A9 or A10 areas. Pretreatment with GR38032F (1 mg/kg IV) did not alter the inhibitory effects of d-amphetamine but did attenuate the excitatory effects of morphine (0.5 mg/kg IV) on A10 DA neurons. These preliminary electrophysiological results support the contention that GR38032F may selectively affect DA hyperactivity without altering basal activity.

339.14

TYPES OF 5-HYDROXYTRYPTAMINE (5-HT) RECEPTORS IN MOLLUSCAN HEARTS. C. D. Bruce*, M. C. Sutter* (SPON: B. R. Sastry). Department of Pharmacology & Therapeutics, The University of British Columbia, Vancouver, Canada, V6T 1W5.

It has been shown that hearts of molluscs are very sensitive to 5-HT. This study examines the contractile response of Saxidomus giganteus hearts to 5-HT, or 5-carboxamidotryptamine (5-CT) a 5-HT_1 agonist, alone and in the presence of an antagonist, methiothepin (MT), and to methysergide (UML), an antagonist with partial agonist properties. The ventricles were suspended at a resting tension of 0.5 g in aerated low Mg seawater at 16° C. Cumulative curves were constructed for force and rate in response to the agents. 5-HT produced an initial small decrease, followed by a marked increase in force (EC_{50}, 2×10^{-7} M) and rate. 5-CT produced only a marked decrease in rate and force (EC_{50}, 9×10^{-10} M) which was antagonised by 5×10^{-7} M methiothepin. UML acted as a partial agonist: an inhibitory effect on force and rate (EC_{50} 2×10^{-8} M) but reduced maximum effect. The biphasic response (initial inhibition followed by excitation) seen with 5-HT suggests that at least 2 types of 5-HT receptors are present in the hearts of Saxidomus. 5-CT produced only a potent inhibitory response (blocked by MT) while UML seems to be a partial agonist.

339.15

NEUROENDOCRINE RESPONSES DEMONSTRATING A FUNCTIONAL UP-REGULATION OF 5-HT RECEPTORS AFTER DESTRUCTION OF SEROTONERGIC NERVE TERMINALS. R.J. Maslowski*, P.A. Rittenhouse*, and L.D. Van de Kar, Loyola Univ. Chicago, Stritch Sch. Med., Dept. Pharmacology, Maywood, Il 60153.

The present studies were designed to determine whether the 5-HT receptors which increase renin and corticosterone (CORT) secretion are pre- or post-synaptic. Rats treated with nomifensine (15 mg/kg, i.p.) to protect catecholamine terminals, were injected bilaterally with 5,7-DHT (75 μg/10 μl/side i.c.v.) to destroy 5-HT nerve terminals. Two weeks later, the rats received an injection of the 5-HT agonist RU24969 (0.2, 1.0, or 5.0 mg/kg, i.p.) or saline, 30 minutes prior to sacrifice. In 5,7 DHT treated rats, there was a shift to the left in the dose-response curve for the effect of RU24969 on CORT release. The minimal dose which significantly increased CORT, decreased from 5.0 mg/kg to 1.0 mg/kg. The dose which produced a significant increase in plasma renin activity/concentration also decreased from 1.0 mg/kg in the vehicle group to 0.2 mg/kg in 5,7-DHT treated rats. In conclusion, the neuroendocrine response to the 5-HT agonist RU24969 was not blocked after the destruction of 5-HT nerve terminals, suggesting that the 5-HT receptors which mediate this response are located postsynaptically. The shift in the dose response curves suggests functional denervation supersensitivity of the 5-HT receptors.

339.16

AGING AND SEROTONIN RECEPTOR BINDING: EVALUATION IN FEMALE FISCHER 344 RATS. R.E. Halpern* and J.M. Lakoski. Dept. Pharmacol., Univ. Texas Med. Branch, Galveston, TX 77550.

An age-related decline in presynaptic 5-HT function has been identified from electrophysiologic recordings of 5-HT dorsal raphe neurons (DRN) in female rats. To identify the mechanisms which may underlie such physiological changes, we evaluated 5-HT receptor binding sites in brain tissue from aged female rats.

Female Fischer 344 rats (Young, cycling 3-4 mo and Old, acyclic 17-18 mo) were ovariectomized (7 days), vehicle implanted (sesame oil) for 48 hr and sacrificed. Brain tissue was rapidly removed, dissected and stored at -80°C until assayed. Radioligand binding studies were carried out using [^{3}H]5-HT (0.05-32 nM), with tissue diluted 1:25 in Tris-HCl buffer; nonspecific binding was determined using 10^{-4}M 5-HT. Scatchard analysis were performed using LIGAND (Elsevier; Coef. Co.> 0.90) and protein values determined by Bio-Rad assay. No alterations in receptor number (Bmax) were detected in Young vs Old prefrontal cortex (PFC; 168 vs 145 pmol/mg prot.) and DRN (166 vs 160 pmol/mg prot.). In contrast, receptor affinity apparently decreased with age (Young vs Old) in the PFC (Kd 10.9 vs 15.4 nM) but not the DRN (Kd 16.8 vs 17.2 nM). These data suggest that age related changes in 5-HT PFC but not DRN receptor binding sites occur in the female rat. Further investigations are evaluating age-induced changes in 5-HT receptor subtypes. Supported by AG 06017.

339.17

HYPERAMMONEMIA LEADS TO INCREASED BRAIN SEROTONIN METABOLISM IN UREASE INFUSED RATS. Mark L. Batshaw, Susan L. Hyman*, Melvyn P. Heyes, John Anegawa*, Joseph T. Coyle, Michael B. Robinson. (SPON: S. Logan) Dept Pediatr, Psychiat and Kennedy Inst, Johns Hopkins School of Medicine, Baltimore, MD 21205

Children with congenital hyperammonemia (HA) develop behavioral alterations including anorexia and changes in activity and sleep. Osmotic minipumps were implanted ip to deliver urease, 4.5 U/100g/d, into halothane anesthetized rats and produce HA. Behavioral alterations included anorexia and increased activity changes; a few rats seized. Mean plasma ammonium levels (n=5 each) just prior to sacrifice at 12, 24 and 48 h. after implant were 1600, 2980, 4900 uM vs sham levels of 77-130. Some nonpolar and large hydrophobic amino acids were increased in cortex. There were 2-4 fold increases in levels of tryptophan, glutamine and 5-HIAA in cortex, $p<0.05$-.001, compared to sham. There were no significant changes in quinolinic acid, the other major pathway for tryptophan metabolism nor in other biogenic amines. We conclude that urease infusion is an apt model of congenital hyperammonemia and that there is an increase in serotonin metabolism that may relate to the neurobehavioral abnormalities in these disorders.

339.18

COMPARISON OF THE ANTI-EMETIC ACTION OF TWO SUBSTITUTED BENZAMIDES, ZACOPRIDE AND BMY 25801 G.L King and V.A. Kieffer* Dept. of Physiology, AFRRI, Bethesda, MD 20814-5145.

These studies were conducted to compare the anti-emetic potency of zacopride (Z; AH Robins) with that of BMY25801 (Bristol-Myers), vs. radiation-induced emesis (RIE) in the ferret. Adult male, castrated ferrets (n = 8 - 10 per group) were given (ip) Z (0.003, 0.03 or 0.3 mg/kg), BMY (3 mg/kg) or vehicle (NaCl) prior to ^{60}Co radiation (2,4 or 6 Gy @ 1 Gy/min). The following emetic parameters were recorded for 2 hrs post-irradiation: latency to first emesis, duration of prodromal phase, duration and number of individual emetic bouts, and number of emetic expulsions (vomitus expelled). 100% of NaCl-irradiated animals vomited at all 3 radiation doses. The ED_{50} for RIE (0.77 Gy; King, Radiat. Res., in press) was increased by 0.3 Z $\geq$ 0.03 Z > 3.0 BMY, but unaffected by 0.003 Z. For each parameter, all responding (vomiting) animals were compared to NaCl-treated controls. Preliminary analysis indicates the following rank order of anti-emetic potency: 0.3 Z $\geq$ 3.0 BMY > 0.030 Z >> 0.003 Z > NaCl-irradiated controls. Depending on administration route, however, Z produced side effects of retching/emesis and defecation in the ferret (King et al., FASEB J 2:A325, 1988). We are currently evaluating BMY for similar effects.

339.19

8-OH-DPAT AND 5-HT3 ANTAGONISTS DIFFER IN EFFICACY VS. MOTION-, XYLAZINE- AND CISPLATIN-INDUCED EMESIS. J.B. Lucot and G.H. Crampton. Dept. Pharmacol., Wright State Univ., Dayton, OH 45435.

Buspirone has been reported to have broad spectrumed antiemetic efficacy in cats. Its site of action required verification by testing 8-OH-DPAT against the same emetic stimuli. 5-HT3 antagonists have been reported to prevent radiation- and cisplatin-induced emesis in ferret. The antiemetic effects of these drugs was tested against three emetic stimuli in cats and compared with 5-HT1A agonists.

The emetic stimuli were: 1) motion in a devise similar to a Ferris wheel, 2) 0.66 mg/kg xylazine SC and 3) 7.5 mg/kg cisplatin IV. 8-OH-DPAT prevented motion sickness with an ED50 of 0.011 mg/kg and xylazine-induced emesis with an ED50 of 0.056 mg/kg. The dose of 0.64 mg/kg significantly decreased the number of emetic events and increased the latency to the first vomit. ICS 205 930 (1 mg/kg SC) completely abolished cisplatin-induced emesis. However, ICS 205 930 (1 and 0.1 mg/kg), MDL 72222 (1 and 0.1 mg/kg) and zacopride (0.01-10 mg/kg) did not prevent motion sickness and ICS 205 930 and MDL 72222 did not prevent xylazine-induced emesis. 5-HT3 antagonists had greater efficacy in preventing cisplatin-induced emesis but stimulation of 5-HT1A receptors had efficacy against several stimuli. The two classes of drugs appear to act through different mechanisms.

339.20

ENDOGENOUS MELATONIN (MEL) REGULATES NORADRENERGIC ACTIVITY IN THE HYPOTHALAMUS OF C3H/HeN MICE. J. M. Fang* and M. L. Dubocovich. (SPON: C. H. Wu). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Activation of MEL receptor sites modulates the activity of norepinephrine (NE) neurons innervating the hypothalamus of C3H/HeN mice (Fang and Dubocovich, FASEB J. 6: A1802, 1988). The role of endogenous MEL in modulating NE activity was assessed by blocking MEL receptor sites with the antagonist luzindole (LUZ, 30 mg/kg, i.p., 30 min). The levels of NE (ng/mg tissue) in the hypothalamus of C3H/HeN mice were determined following inhibition of NE synthesis with α-CH_3-p-tyrosine (α-MpT, 300 mg/kg, i.p., 2 h) by HPLC with EC detection. At noon, when the levels of endogenous MEL are low, LUZ did not affect the turnover of NE [Noon: Control: 2.82 ± 0.1 (n=10), α-MpT: 2.17 ± 0.06 (n=10), α-MpT plus LUZ: 2.14 ± 0.26 (n=4)]. 6-Cl-MEL (30 mg/kg, i.p., 30 min) reversed the α-MpT induced-depletion of NE [α-MpT plus 6-Cl-MEL: 2.69 ± 0.1 (n=10), $p<0.001$]. This effect was blocked by LUZ [α-MpT plus 6-Cl-MEL plus LUZ: 2.27 ± 0.06 (n=9), $p<0.005$]. At midnight, when the levels of MEL are high, LUZ significantly decreased the levels of NE after treatment with α-MpT, suggesting activation of MEL receptor sites by endogenous MEL [Midnight: Control: 3.39 ± 0.2 (n=10), α-MpT: 2.82 ± 0.15 (n=10), α-MpT plus LUZ: 2.38 ± 0.12 (n=7), $p<0.05$]. We conclude that the activation of MEL receptor sites in C3H/HeN mouse brain by endogenous MEL inhibits NE activity leading to decrease in NE release in the hypothalamus. Supported by grant DK 38607.

340.1

DOPAMINE AGONISTS MAINTAIN MAGNESIUM INDUCED CONDITIONED PLACE PREFERENCE. S.I. Lawley and K.M. Kantak. Lab. of Behav. Neurosci., Dept. of Psychol., Boston University, Boston, MA 02215.

Reinforcing properties of magnesium (Mg^{2+}) were studied using conditioned place preference (CPP) in mice. It is well known that stimulants will support CPP. Previous studies indicate that magnesium chloride ($MgCl_2$) will act as a reinforcer in the CPP paradigm at a dose of 15 mg/kg. We used a substitution paradigm in the CPP procedure to test the effects of various drugs on Mg^{2+} reinforced behavior. Only animals which showed a greater than 50% shift in preference were used in the substitution procedure. In these experiments 64% of the animals showed a change in preference following conditioning. The indirect dopamine (DA) agonists, amphetamine and cocaine, maintained Mg^{2+} conditioning while the DA blocker haloperidol significantly attenuated it. Pentobarbital which is known not to bind the DA receptor also maintained Mg^{2+} conditioning. Results implicate involvement of Mg^{2+} in the DA theory of reward.

340.2

THE EFFECTS OF INTRA-VTA INFUSIONS OF MORPHINE AND DYNORPHIN(1-13) ON MALE SEXUAL BEHAVIOR. J.B. Mitchell and J. Stewart, Center for Studies in Behavioral Neurobiology, Dept. of Psychology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

Data suggests that the mesolimbic DA system is involved in mediating sexual arousal (Baum & Starr, Pharm. Biochem. & Behav., 13:175, 1980; Cagguila et al., Brain Res., 111:321, 1976). Morphine is known to increase the activity of dopaminergic cells (Matthews & German, Neurosci., 11:617, 1984; Gysling & Wang, Brain Res., 277:119, 1983), and when applied to the region of mesolimbic DA cell bodies, the VTA, increases locomotor activity (Joyce & Iversen, Neurosci. Let., 14:207, 1979; Vezina & Stewart, Pharm. Biochem. & Behav., 20:925, 1984), and feeding (Hamilton & Bozarth, Neurosci. Abs., 13:412, 1987). The opioid peptide, dynorphin, applied to the VTA also increases feeding (Hamilton & Bozarth, 1987).

Castrated males, maintained on behaviorally subthreshold doses of testosterone, were tested for sexual behaviors after intra-VTA infusions of 0.1 to 30 nmoles morphine, or of 0.03 to 3 pmoles dynorphin(1-13). Both morphine and dynorphin produced dose-dependent increases in the number of males that mounted and increased the display of female-directed behaviors, such as pursuit of the female, anogenital exploration, and partial mounts. To investigate the effect of dynorphin on mesolimbic dopamine metabolism, different groups of animals were sacrificed after intra-VTA infusions of saline, 10 nmoles morphine, or 0.3 pmoles dynorphin(1-13) and DA and DOPAC levels in the nucleus accumbens and medial frontal cortex estimated using HPLC-EC. Morphine, but not dynorphin, increased the concentration of DOPAC and the DOPAC/DA ratio in the nucleus accumbens.

These results suggest that the mesolimbic dopamine system is involved in mediating sexual arousal, but that other non-dopaminergic elements in the VTA, perhaps accessed by kappa-opioid receptors, are also involved in sexual behaviors.

340.3

PLASMA HOMOVANILLIC ACID (HVA) CHANGES DURING TESTOSTERONE OR NANDROLONE ADMINISTRATION IN HUMANS. C.J. Hannan, Jr., T.M. Kettler,* K.E. Friedl* and S.R. Plymate.* Clinical Investigation Dept, Madigan Army Med Ctr, Tacoma WA 98431.

An association of anabolic steroid administration with psychiatric symptoms prompted our examination of proposed biochemical markers of psychosis during steroid administration. Twenty-five male subjects received 6 weekly IM injections of 100 or 300 mg testosterone enanthate (Te), or 100 or 300 mg nandrolone decanoate (Nan). Two blood samples were drawn on different days before drug administration, and two were drawn during the 6th wk after the final drug dose. Serum levels of HVA and 5-hydroxyindoleacetic acid (5-HIAA) were determined by HPLC with dual electrode detection and separated with two mobile phases to confirm the purity of the eluted compounds. Increases in plasma HVA were found in both Nan groups but not in the Te subjects. Plasma HVA is an indicator of tissue dopamine turnover.

Metabolite* (ng/ml)	100 mg TE n=7	300 mg TE n=5	100 mg Nan n=6	300 mg Nan n=7
Δ HVA	0.6±1.2	-1.0±1.3	3.2±1.8†	2.0±0.6†
Δ 5-HIAA	-2.5±3.0	0.1±3.0	0.9±3.6	-3.2±4.3

*Mean changes (post-pre)±SE † = p<0.05 by Wilcoxon

The large proportion of plasma HVA which originates from CNS metabolism of dopamine has been suggested to explain its correlation with severity of psychosis in psychiatric patients. The demonstrated alteration in dopamine metabolism associated with Nan provides a mechanism to explain reported altered behavior in some anabolic steroid users.

340.4

THE PRIMING PHENOMENON IN THE EXPRESSION OF DOPAMINE RECEPTOR SUPERSENSITIVITY: DOSE AND TEMPORAL DEPENDENCY. M. Morelli & G. Di Chiara. Inst. Exp. Pharm. & Tox. Univ. Cagliari, Italy.

We have recently shown in rats unilaterally lesioned with 6-hydroxydopamine (6-OHDA) from 14 days, that the expression of D-1 receptor supersensitivity depends upon previous "priming" with a DA receptor agonist. In this study we examined the temporal and the dose-dependency characteristics of "priming". Male Sprague-Dawley rats lesioned in the left medial forebrain bundle (MFB) with 6-OHDA were used throughout all the experiments. Administration of the D-1 receptor agonist SKF38393 (2mg/kg) failed to induce contralateral turning (c.t.) in naive rats lesioned from 14 days, but produced an intense contralateral turning in naive rats lesioned from 90 days. A single administration of the D-1/ /D-2 receptors agonist apomorphine (0.1 mg/kg, 3 days before) which produced c.t. by itself, made SKF38393 very active in inducing c.t. in 14 days lesioned rats. The "priming" induced by apomorphine develops with time, in fact administration of SKF38393 three hours after apomorphine, failed to elicit c.t..Like apomorphine, also the D-2 receptor agonist LY171555 and SKF38393 itself induced "priming" in 6-OHDA lesioned rats from 14 days. This "priming" however was strictly dependent from the dose of the drug used. Moreover "priming" with any of the mentioned drugs was ineffective in rats lesioned from 7 days. The results indicate that efficacy of "priming" depends from the time of "priming" and from the dose of the drug used for "priming".

340.5

LESIONS OF THE SUBSTANTIA INNOMINATA UNMASK AN INHIBITORY EFFECT OF APOMORPHINE ON ACOUSTIC STARTLE. C.B. Sananes, J.M. Hitchcock, J.B. Rosen, M.J.D. Miserendino & M. Davis. Dept of Psychiatry, Yale University, Ribicoff Research Fac., Conn. Mental Health Ctr., New Haven, CT, 06508.

Dopamine receptor subtype agonists have opposite effects on the acoustic startle reflex. D1 agonists increase startle whereas D2 agonists depress it. The net effect of the mixed D1 and D2 agonist apomorphine is an enhancement of startle. However, this effect can be reversed into inhibition by pretreatment with the D1 antagonist SCH 23390. The present study sought to determine whether various dopamine projection areas are involved in apomorphine's effect on startle.

Rats were given bilateral electrolytic lesions of either the nucleus accumbens, the central nucleus of the amygdala, the prefrontal cortex or the substantia innominata. Following recovery, animals were tested for the effects of apomorphine (0.4 mg/ kg s.c.) and saline on acoustic startle. Half of the animals were tested with apomorphine first, and the other half with saline first. Sham-lesioned and unoperated groups were also tested.

Lesions of the nucleus accumbens, the central nucleus of the amygdala, or the prefrontal cortex had no effect on apomorphine's enhancement of startle. In contrast, lesions of the substantia innominata changed the usual excitatory effect of apomorphine into an inhibitory one. Thus, lesions of the substantia innominata,which receives dopamine projections from A8 and A10, had the same effect as pretreatment with a D1 antagonist where the D1 excitatory effect was blocked, thereby unmasking an underlying inhibitory effect of apomorphine. These data suggest that the D1and D2 effects of apomorphine on startle are independent and have different anatomical loci of action.

340.6

DIFFERENTIAL EFFECTS OF SELECTIVE DOPAMINE AGONISTS AND ANTAGONISTS ON STARTLE ELICITED ELECTRICALLY FROM THE BRAINSTEM. K.R. Melia* & M. Davis (SPON: W.P. Jordan). Dept. of Psychiatry, Yale Univ., Ribicoff Res. Fac., CMHC, New Haven, CT. 06508.

The mixed D1, D2 dopamine agonist, apomorphine, is known to increase the amplitude of the acoustic startle reflex. Pretreatment with the selective D1 antagonist, Sch 23390, reverses the usual excitatory effect of apomorphine into an inhibitory one. Pretreatment with the D2 antagonist, sulpiride, augments the excitatory effect of apomorphine. These data suggest that activation of D1 dopamine receptors increases startle whereas activation of D2 dopamine receptors decreases startle. Acoustic startle is mediated by a serial neural circuit consisting of the ventral cochlear nucleus (VCN), the ventral lateral lemniscus (VLL), the reticularis pontis caudalis (RPC), and the spinal cord. By eliciting startle electrically at different points along this pathway the present study sought to determine where D1 and D2 receptor activation ultimately alters neural transmission so as to have opposite behavioral effects.

Previous studies have shown that apomorphine increases startle elicited by an acoustic stimulus but decreases startle elicited by electrical stimulation of the VCN. In the present study the D2 agonist, Lilly 171555, was found to depress startle elicited both acoustically and electrically from the VCN. The inhibitory effect of the D2 agonist was further localized since Lilly 171555 did not depress startle elicited electrically from the RPC, the last supraspinal synapse of the startle pathway. In addition, preliminary data indicate that the D1 antagonist, Sch 23390, depresses acoustically elicted startle but not startle elicited electrically from the VCN.

These results suggest that the excitatory effect of apomorphine is mediated by its D1 activity and impinges upon the startle pathway at or before the VCN while apomorphine's inhibitory effect is mediated by its D2 activity and impinges upon the startle pathway supraspinally but downstream from the VCN.

340.7

EFFECTS OF ALPHA-METHYL-PARA-TYROSINE ON THE MAINTENANCE OF CONDITIONED PLACE PREFERENCE. N. Hiroi* and N.M. White. Department of Psychology, McGill University, Montreal, Canada.

When dopaminergic function in nucleus accumbens is disrupted on the training days in the conditioned place preference (CPP) paradigm, CPPs for amphetamine or food are not observed, suggesting that this neurochemical mediates some aspect of primary reward. The purpose of the present study was to determine if normal dopamine function is necessary on the test day to observe a preference previously conditioned with amphetamine. Rats were given 12 training days in a CPP apparatus, including 6 pairings of 2 mg/Kg d-amphetamine with one part of the apparatus and 6 pairings of saline with the other part in a counterbalanced manner. Bilateral injections of 80 ug of a-MPT into nucleus accumbens prior to testing had no effect on the CPP observed, compared to animals receiving saline or no injections. In a control experiment, bilateral injections of the same dose of a-MPT into nucleus accumbens significantly suppressed the locomotor activity produced by 2 mg/kg of d-amphetamine without causing motor impairment, suggesting that this treatment blocks dopamine release. Although there is evidence suggesting that dopamine in nucleus accumbens mediates some aspect of primary reward, the present result suggests that the memory of the primary reward, which is expressed as a place preference on the test day, is not mediated by dopamine release.

340.8

DOPAMINE D1 AND D2 RECEPTORS AND LOCOMOTOR ACTIVATION ELICITED FROM THE NUCLEUS ACCUMBENS OF RATS. J.K.Dreher and D.M.Jackson. Pharmacology Department, Sydney University, N.S.W. 2006, AUSTRALIA.

While it is well documented that the injection of DA agonists into the accumbens (Acb) of rats produces locomotor activation, it is less clear which DA receptor subtype is important and whether there is any interaction between them. Early studies suggested that D1 receptors played a role, with cholera toxin, dibutyryl cyclic-AMP and SKF38393 (SKF) producing excitation after direct injection. In the present study, (RS)-SKF (1 to 10 µg/side) produced dose-dependent long-lasting (up to 12 h) stimulation after local application, characterized by co-coordinated locomotion, sniffing and some rearing. Only the R-enantiomer was active. SKF stimulation (10 µg) was blocked by SCH23390 (0.5 mg/kg, ip) and spiperone (0.1 mg/kg), but not by ketanserin. The D1 agonist, CY208-243 (CY) was only slightly active (up to 8 µg), and the activity lasted for 2 h. Quinpirole (quin, 0.3 to 3.0 µg) also increased activity for about 2 h. DA depletion blocked the stimulant effects of SKF, CY and quin. However, stimulation was produced in these DA-depleted rats when SKF plus quin or CY plus quin was injected into the Acb. The activity consisted of coordinated forward locomotion which was generally devoid of stereotypies. The stimulation lasted for about 5 h (SKF/quin) or 2 h (CY/quin). In rats with their DA stores intact, SKF (or CY) plus quin had an at least additive effect on stimulating activity. The data clearly show that both D1 and D2 receptors play an interactive and important role in mediating stimulation elicited by DA agonists in the Acb. SUPPORTED BY THE NH & MRC.

340.9

EFFECTS OF STIMULATION AND BLOCKADE OF DOPAMINE RECEPTOR SUBTYPES ON THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE. R. L. Barrett* and J. B. Appel. *Behavioral Pharmacology Laboratory Department of Psychology University of South Carolina, Columbia, SC 29208 USA.*

The involvement of dopamine (DA) receptor subtypes in the behavioral (subjective) effects of cocaine was studied in rats which were trained to discriminate 10 mg/kg of this substance from saline. In substitution tests, cocaine did not generalize to the selective D_1 agonist SK&F 38393 (5.0 - 15.0 mg/kg); however, significant amounts of drug-appropriate responding did occur following relatively high doses of *d*-amphetamine (0.25 - 1.0 mg/kg) and the D_2 agonist LY 171555 (0.05 - 0.25 mg/kg). In combination tests, the D_1 antagonist SCH 23390 (0.0625 - 0.5 mg/kg) as well as the D_2 antagonists spiperone (0.25 - 0.5 mg/kg) and haloperidol (0.0625 - 1.0 mg/kg) attenuated, but did not completely block the cocaine cue. These data suggest that DA neuronal systems probably play a role in the *in vivo* effects of cocaine, but that the stimulus properties of this compound involve mechanisms that are more complex than direct activation of D_1 *or* D_2 receptors (e.g., stimulation of both D_1 *and* D_2 receptors).

340.10

DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE AND PROCAINE. P.B. Silverman. Dept. of Psychiatry, Univ. of Texas School of Medicine, Houston, TX 77030.

The abuse potential of cocaine and other psychomotor stimulants has been attributed to dopaminergic activity as has the discriminative stimulus that results from cocaine administration. Others have shown that some local anesthetics, including procaine, have stimulus properties with similarity to those of cocaine. There is, however, little evidence of dopamine agonist activity by locals. The work here compared the stimulus properties of cocaine and procaine in rats trained to discriminate one of these compounds from saline in a two lever, food reinforced operant procedure. The intent was to shed light on the apparent inconsistency concerning dopamine mediation of the stimuli.

The results to date suggest that while cocaine- and procaine-induced stimuli are, indeed, somewhat similar, only other psychomotor stimulants generalized completely to cocaine (and not to procaine) and haloperidol more effectively blocked cocaine than procaine recognition.

340.11

GENOTYPIC SEPARATION OF SUSCEPTIBILITY TO THE MOTOR ACTIVITY-STIMULATING ACTION AND REINFORCING ACTION OF COCAINE. T.W. Seale, L. Logan*, W. Landrum* and J.M. Carney*. Dept. of Pediatrics, Univ. Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK 73190 and Dept. of Pharmacology, Univ. Kentucky Sch. Medicine, Lexington, KY 40536.

Little is known about the genetic determinants influencing susceptibility to the behavioral actions of cocaine. We have utilized inbred mice as a model system to assess the role of genotype in drug-seeking behavior (DSB). Conditioned place preference (CPP) was used as a behavioral marker for cocaine-induced DSB. Conditioning to environmental cues was carried out in a chamber consisting of two compartments, one black with a wire mesh floor and cedar smell and one white with a wood chip floor and pine smell. Mice (n=10 for each treatment) were injected with vehicle or cocaine and placed in the conditioning chamber for 30 mintues. CPP was assessed 24 hours following 1 to 5 daily conditioning trials. Two strains of mice, C57BL/6J and BALB/cByJ, showed significant ($p < 0.005$), dosage dependent, trial dependent, stereospecific induction of CPP to the innately less preferred compartment. In contrast, the motor activity-stimulating effects of cocaine differed markedly in the two mouse strains. C57BL/6J showed the expected dosage-dependent stimulation of motor activity (ED_{100} about 18 mg/kg ip). Cocaine failed to induce motor activity stimulation in BALB/cByJ at any dose. These data indicate that genotype can influence the inherent behavioral responsiveness of an animal to cocaine. Relative susceptibility to its motor-activity effects does not predict inherent susceptibility to its reinforcing properties.

340.12

LEGAL STIMULANTS MIMIC AND POTENTIATE THE DISCRIMINATIVE EFFECTS OF COCAINE. R.D. Harland*, D.V. Gauvin*, and F.A. Holloway (SPON: T. Steele). Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.

The effects of caffeine and caffeine-phenylethylamine combinations upon the discriminative and rate-altering effects of cocaine were examined in rats. Twelve male Sprague-Dawley rats were trained in a two-choice, food reinforced, drug discrimination task with 10 mg/kg cocaine and saline as discriminative stimuli. Stimulus generalization tests with cocaine resulted in a dose-related increase in cocaine-appropriate responding and variable decreases in response rates. Caffeine also engendered a dose-related increase in cocaine-appropriate responding (but only partial generalization at the highest dose tested) and a biphasic dose-effect curve for response rate. Caffeine potentiated the discriminative stimulus properties of cocaine, with isobolographic analysis characterizing the interaction as simple additivity. Caffeine's effects upon the rate-reducing effects of cocaine resulted in a biphasic interaction pattern. Rats were also tested with a wide range of cocaine doses and several dose combinations of caffeine, ephedrine, and phenylpropanolamine (CEP). The CEP combinations resulted in criterion generalization at high doses. All drugs produced response rate decrements at high doses. These data indicate that certain look-alike stimulants (i.e., CEP combinations) mimic the cocaine cue.

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340.13

SOME EFFECTS OF TYPICAL AND ATYPICAL NEUROLEPTICS ON NONDEPRIVED RATS LICKING SUCROSE SOLUTIONS.
S.E. Gramling*, H.F. Villanueva & J.H. Porter. (SPON: B.F. Kilpatrick). Dept. of Psych., Virginia Commonwealth Univ., Richmond, VA 23284

The stereotypic nature of the lick response has proved a useful tool in separating the motor effects from the hedonic effects of neuroleptics. The present experiment extends this research by assessing the effects of typical vs atypical neuroleptics on several measures of rats' licking.

Thirty nondeprived rats licked a 32% sucrose solution at stable rates and were then randomly assigned to one of four acute neuroleptic dosing regimes. The treatment groups were Haloperidol (HAL: 0.075, 0.15, 0.3 mg/kg, ip.) Pimozide (PIM: 0.5, 1.0, 1.5, mg/kg, ip.) Clozapine (CLOZ: 1.25, 2.5, 5.0, mg/kg, ip.) and Sulpiride (SULP: 6.25, 12.5, 25.0 mg/kg, ip.). Lick rate, duration and ILI data were collected via laboratory computer.

Overall, all four groups of rats exhibited a significant dose related decrease in response rate. Rats treated with either of the typical neuroleptics (HAL, PIM) exhibited monotonic rate decreases, whereas the CLOZ and SULP (atypical neuroleptics) treated rats exhibited a nonmonotonic pattern. The measures lick duration and ILI also differentiated these neuroleptics.

PAIN MODULATION: BIOGENIC AMINES

341.1

DEPLETION OF SPINAL CORD NOREPINEPHRINE ATTENUATES ANTINOCICEPTION INDUCED BY INTRATHECAL 5'-N-ETHYLCARBOXAMIDE ADENOSINE AND NUCLEUS RAPHE MAGNUS STIMULATION.
Aran, S. and Proudfit, H.K. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, Il 60680.

We examined the capacity of spinal cord norepinephrine (NE) depletion to attenuate the antinociception produced by the interaction between intrathecal (IT) 5'-N-ethylcarboxamide adenosine (NECA) and release of endogenous NE produced by nucleus raphe magnus (NRM) stimulation.

Animals received an IT injection of either vehicle or 6-OHDA. Twenty one days after surgery baseline tail flick latencies (TFLs) were determined and a stimulus response curve was generated for electrical stimulation of the NRM. Animals were then given an IT injection of a subeffective dose of NECA. TFLs were determined 30 min after drug injection and stimulus-response curves were constructed. NRM stimulation produced significant increases in TFLs both before and after NECA injection in animals pretreated with vehicle. In animals injected with 6-OHDA, NRM stimulation failed to increase TFLs. 6-OHDA decreased NE levels to 1.1 % of control values but did not alter 5-HT levels.

These data suggest that endogenous NE, released by NRM stimulation, interacts synergistically with NECA to produce antinociception. This work was supported by USPHS Grant DA03980.

341.2

MDL 26,764, A NON-NARCOTIC ANALGESIC WITH α_2-ADRENERGIC AGONIST-LIKE PROPERTIES.
F.P. Miller, D.L. Braun, H.J. Ketteler and A.A. Carr. Merrell Dow Research Institute, Cincinnati, OH 45215.

Analgesic activity of MDL 26,764 {1-piperidineethanol,α-(4-fluorophenyl)-4-[(4-fluorophenyl)hydroxymethyl]-} was assessed against acetic acid-induced writhing in mice and rats. ED50 values, determined 30 min after sc administration, were 1.85 and 4.73 mg/kg, respectively. This activity was present for at least 4 hrs in mice (ED50 values of 8.73 mg/kg sc and 27.6 mg/kg po). MDL 26,764 also produced analgesic activity after intraventricular (ED50 19.5 µg/mouse) or intrathecal (ED50 14.7 µg/mouse) administration. In mice, MDL 26,764 increased response latency in the tail immersion test, but was ineffective in altering response latency in the hot plate test. The analgesic activity of MDL 26,764 was completely antagonized by systemic administration of the selective α_2-antagonist, idazoxan(I), whereas benextramine, an α_2-antagonist that does not cross the blood-brain barrier, and the opiate antagonist, naloxone, were ineffective. Colonic motility in mice, known to be altered by α_2-agonists, was inhibited by MDL 26,764; this inhibition was reversed by I. These results indicate that MDL 26,764 is a centrally acting, non-narcotic analgesic with a mechanism of action dependent primarily on an agonist-like effect on α_2-adrenergic receptors.

341.3

INHIBITION OF NSAID-INDUCED ANTINOCICEPTION IN MICE BY ALPHA-2 ADRENERGIC RECEPTOR BLOCKADE.
E. Baizman*, N. Beglin*, D. Koonz* and D. Luttinger. Dept. of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, NY 12144.

Central alpha-2 adrenergic blockade has been reported to attenuate antinociception produced by morphine (e.g. Camarata and Yaksh, Brain Res. 1985). We now report that the antinociceptive effects in the mouse acetylcholine-induced writhing test of two non-steroidal antiinflammatory analgesics (NSAIDs), naproxen and zomepirac, are also attenuated by alpha-2 adrenergic receptor antagonists.

Male Swiss-Webster mice were treated with receptor antagonists (s.c.) 5 min. prior to administration of an NSAID (i.v.) then challenged with acetylcholine (ACh; 3.2 mg/kg i.p.) or phenyl-p-quinone (PPQ; 1 mg/kg i.p.). S.C. administration of yohimbine (3 mg/kg) or idazoxan (RX781094; 1 mg/kg) shifted the naproxen dose-response curve to the right. Antinociceptive effects of another NSAID, zomepirac, were also inhibited by yohimbine pretreatment. The writhing response to ACh was not affected by yohimbine pretreatment, suggesting that nociceptive thresholds to ACh were not altered. Yohimbine (3 mg/kg, s.c.) did not alter the antinociceptive effects of naproxen in the PPQ writhing test.

These data suggest that alpha-2 adrenergic antagonists can modify antinociception induced by NSAIDs. However, these effects may be dependent on the nociceptive stimulus used. It is unclear whether this is due to differences in intensity of nociceptive stimulus or to mechanisms of inducing nociception.

341.4

VISCERAL NOCICEPTION: INHIBITION BY SPINAL α_2-ADRENOCEPTORS.
R.M. Danzebrink and G.F. Gebhart. Department of Pharmacology, The University of Iowa, Iowa City, Iowa, 52242.

The antinociceptive effects of the intrathecal administration of the α_2-adrenoceptor agonists clonidine, ST-91, and tizanidine, the nonselective α-adrenoceptor agonist norepinephrine, and the β-adrenoceptor agonist isoproterenol were examined in awake, unanesthetized rats. Colorectal distension, the noxious visceral stimulus employed, elicits a vigorous pressor response and contraction of abdominal and hindlimb musculature (a visceromotor response). Chronic intrathecal and arterial catheters were implanted. At the time of experimentation a distensible, latex balloon was inserted nonsurgically via the anus into the descending colon and rectum, and distensions were given 3 minutes apart. All drugs were administered in equal volumes, 7.5 µl, followed by a 7.5 µl flush of saline. Cumulative doses were given at 12 minute intervals. The α-adrenoceptor agonists produced a dose-dependent attenuation of the pressor and visceromotor responses. Pretreatment with the α_2-adrenoceptor antagonist yohimbine antagonized the effects produced by clonidine, ST-91, and tizanidine, whereas the effects produced by norepinephrine were not significantly altered. The β-adrenoceptor agonist isoproterenol did not attenuate the pressor or visceromotor response to colorectal distension. These results demonstrate that spinal α_2-adrenoceptors, and not β-adrenoceptors, mediate antinociception to noxious visceral stimulation.

341.5

DEMONSTRATION OF A UNIQUE ^{3}H-5-HT BINDING SITE IN RAT SPINAL CORD. F.P. Zemlan, E.F. Schwab and R.M. Murphy. Lab of Geriatric Research, and Dept. of Physiology, Univ. of Cincinnati Col. of Med., Cinti, OH 45267-0555.

The present studies identify a new high affinity ^{3}H-5-HT binding site in spinal cord which has a unique pharmacologic profile not observed in frontal cortex.

The density of 5-HT 1A, 1B and 1C receptors in rat frontal cortex and spinal cord was determined in saturation (^{3}H-5-HT, ^{3}H-8-OH-DPAT, ^{3}H-mesulergine) and competition (10^{-12} to 10^{-3} M) studies. In cortex, competition studies (^{3}H-5-HT) employing selective 1A masks (8-OH-DPAT, buspirone), 1B mask (RU24969) and 1C mask (mesulergine) indicated that 38±5% of ^{3}H-5-HT binding sites were of the 1A subtype, 47±5% 1B and 17±2% 1C subtypes, apparently accounting for all cortical 5-HT receptors. Competition studies in cortex confirmed these estimates. In spinal cord, the same experimental techniques identified that 25±3% of ^{3}H-5-HT binding sites were of the 1A subtype and 33±3% were 1B receptors. Subsequent competition studies indicated that the remaining 42% of specific high affinity ^{3}H-5-HT binding in spinal cord (Ki=6±1 nM) was not to 5-HT1C, 5-HT2 or 5-HT3 receptors. More extensive pharmacologic characterization of this unique ^{3}H-5-HT binding site is in progress.

341.6

SEROTONIN RELEASES ADENOSINE FROM PRIMARY AFFERENT NERVE TERMINALS IN THE SPINAL CORD: POSSIBLE INVOLVEMENT IN SPINAL ANTINOCICEPTION. M.I.Sweeney, T.D. White and J. Sawynok. Dept. of Pharmacology, Dalhousie Univ., Halifax, Nova Scotia, Canada. B3H 4H7.

Antinociception produced by intrathecal morphine and serotonin (5-HT) is blocked by adenosine receptor antagonists suggesting that adenosine release may mediate this antinociception. Morphine releases adenosine from the spinal cord in vitro and in vivo; the purpose of the present study was to determine whether 5-HT also releases adenosine from the spinal cord. Release of adenosine evoked by 5-HT from spinal cord synaptosomes was determined by HPLC with fluorescence detection of etheno-adenosine. In some cases, rats were pretreated with capsaicin either as neonates or adults and used in adenosine release studies 17-20 weeks or 1 week later, respectively. 5-HT (50 μM) increased the release of endogenous adenosine from dorsal, but not ventral, spinal cord synaptosomes. This release was reduced by the 5-HT receptor antagonist methysergide, removal of Ca^{2+} from the medium, inhibition of ecto-5'-nucleotidase, and both methods of capsaicin pretreatment. These results suggest that activation of 5-HT receptors on small diameter primary afferent terminals produces a Ca^{2+}-dependent release of a nucleotide which is converted extracellularly to adenosine. This adenosine may contribute to the spinal antinociceptive effect of 5-HT.(Supported by MRC Canada)

341.7

LAMINAR DISTRIBUTION OF RAPHESPINAL FIBERS IN THE RAT LUMBAR DORSAL HORN DEMONSTRATING SEROTONIN-LIKE IMMUNOREACTIVITY. S.L. Jones and A.R. Light. Dept. of Physiology, Univ. of N. Carolina, Chapel Hill, NC 27599.

The nucleus raphe magnus (NRM) has been implicated in the centrifugal modulation of spinal nociceptive transmission. Serotonergic raphespinal projections have been demonstrated using the retrograde tracer horseradish peroxidase in conjunction with immunohistochemistry, however, the laminar distribution of serotonin-containing raphespinal fibers and terminals within the dorsal horn has not been examined. The purpose of this study was to address this issue by using the anterogradely transported lectin, *Phaseolus vulgaris*-leucoagglutinin (PHA-L) coupled with immunohistochemistry. Male, Sprague-Dawley rats were anesthetized with an IM injection of ketamine/xylazine. Microinjections of PHA-L (10%, 0.2μl) were made into the ventromedial medulla and the rats were allowed to recover. After 4 weeks, they were perfused transcardially and the spinal cord tissue was removed and reacted with PHA-L antibody tagged with Texas Red and serotonin antibody tagged with fluorescein. The results to date, indicate that cell bodies in the medial medulla project to all laminae in the lumbar dorsal horn and ventral horn; many fibers were found labeled with PHA-L in a golgi-like fashion. A small percentage (4.8±1.3%, n=5) of PHA-L terminations labeled with serotonin immunoreactivity were found in each of the laminae I-VI, VII-X. Supported by DA04420 and DA05341

341.8

INNERVATION OF IDENTIFIED PRIMATE SPINOTHALAMIC TRACT NEURONS: ULTRASTRUCTURE OF SEROTONERGIC AND OTHER SYNAPTIC PROFILES. C.C. LaMotte, S.M. Carlton, C.N. Honda, D.J. Surmeier, and W.D. Willis. Section of Neurosurgery, Yale Univ. Sch. Med., New Haven, CT 06510 and Marine Biomed. Inst. Univ. Texas Med. Branch, Galveston, TX 77550.

We have combined intracellular staining of identified spinothalamic tract (STT) neurons with immunocytochemistry to define the terminations of primary afferent and modulating systems onto these cells in the lumbosacral cord of anesthetized monkeys (M. fascicularis). The STT cells were antidromically activated from the contralateral thalamus, and after physiological characterization were intracellularly marked with HRP. Following perfusion and post fixation with 2.5% paraformaldehyde, serial vibratome sections were reacted for HRP and then immunohistochemically labeled for serotonin (5-HT) using the PAP method. Sections were flat embedded for light microscopic reconstruction and then cut for EM.

The presently described neuron was a WDR neuron responsive to activation of low and high threshold cutaneous afferents innervating the foot. The soma was located in the lateral half of lamina V, with an extensive dendritic tree. Terminal types innervating this cell were of the same type and in closely corresponding frequency to those found on two other lamina V STT neurons we have studied previously. Over 50% were R terminals; the remaining contained large or small dense core vesicles, or flat vesicles. Most serotonergic terminals were also R type, although 5HT terminals containing either small dense cores (D) or large granular vesicles (L) were also present. L and D terminals were found on the soma of the STT cell but rarely on its proximal dendrites within lamina V. (Supported by NIH grants NS13335, NS11255).

341.9

DISTRIBUTION OF SPINOMESENCEPHALIC TRACT (SMT) CELLS AND CHEMICALLY IDENTIFIED TERMINALS IN THE RAT SPINAL CORD. R.P. Yezierski, K.E. Miller and C.M. Mendez*. Dept. of Neurological Surgery, Univ. of Miami, Sch. of Med., Miami, Fl. 33136.

Previous studies have shown that the distribution of SMT cells in the rat spinal cord overlaps with several areas receiving input from various putative neurotransmitters including serotonin (5HT), substance P (SP) and leu-enkephalin (l-enk). In the present study the technique of retrograde transport was combined with immunohistochemistry to study the relationship between SMT cells and chemically identified terminals in the rat spinal cord. Injections of fluorescent tracers (DAPI or Fluorogold) were made at different locations in the midbrain. Following survival times of 4-11 days animals were perfused sequentially with saline and 4% paraformaldehyde. Spinal cords were cut on a cryostat and processed for 5HT, SP and l-enk immunofluorescence.

Results of this study have shown 5HT, SP and l-enk varicose fibers are in close apposition with SMT cells in the superficial laminae of the dorsal horn, nucleus proprius, the lateral neck of the dorsal horn, lamina X and the lateral spinal nucleus. Close apposition of immunoreactive fibers were observed on large and small somata and on dendritic profiles.

This work was supported by NIH grant NS19509 and by funds from the Miami Project Foundation.

341.10

SEROTONERGIC MEDIATION OF SPINAL ANALGESIA AND ITS INTERACTION WITH NORADRENERGIC SYSTEMS. I. Nakagawa,* K. Omote,* L.M. Kitahata and J.G. Collins. Dept. of Anesthesiology, Yale Univ. New Haven, CT 06510

Serotonin (5HT) was administered intrathecally onto cat spinal cords while recording from WDR neurons in order to evaluate its depression of noxiously evoked activity and the interaction of serotonergic and noradrenergic systems. 500ug(n=8), 1000ug(n=8) and 2000ug(n=22) of 5HT produced significant suppression of the mean noxiously evoked activity (radiant heat) of WDR neurons in the dorsal horn of the spinal cord. Intravenous administration of methyseride (nonselective 5HT antagonist, 1 or 2mg, n=5) or yohimbine (alph2-adrenergic antagonist, 0.5 or 1.0mg/kg, n=4) produced a significant antagonism of the effects of 2000ug of 5HT. In contrast to the effects of methysergide and yohimbine, intravenous administration of corynanthine (alph1-adrenergic antagonist, 0.25 or 0.5mg/kg, n=4) or naloxone (0.1 or 0.2mg, n=4) had no effect upon the antinociceptive effects of 5HT. The combination of low dose 5HT (250ug) and low dose clonidine (5ug) produced a supra-additive suppression. These data support the concept that noradrenergic systems, possibly through an alpha2-adrenergic mechanism, are involved in the modulation of spinal WDR neurons by 5HT.

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341.11

INTERACTION OF SELECTIVE OPIATE RECEPTOR AGONISTS AND CLONIDINE IN ACUTE SPINAL CATS. K. Omote,* I. Nakagawa,* L.M. Kitahata, J.G. Collins. (Spon: J. Heavner) Dept. of Anesthesiology, Yale Univ. School of Medicine, New Haven, CT 06510

This study examined the potential antinociceptive activity of the combination of the mu-selective agonists DAGO or morphine or the delta selective agonist DPDPE with clonidine. We recorded extracellularly from discriminated single WDR neurons activated by noxious radiant heat in decerebrate, spinally transected cats. Following control studies, sub-effective doses of DAGO (1 or 1.5ug), DPDPE (30ug), or morphine sulfate (25ug) were applied gently onto the cord. As combination doses, each sub-effective dose described above was combined with the sub-effective doses of 5ug of clonidine. Sub-effective doses of DAGO, DPDPE, morphine or clonidine alone did not significantly suppress noxiously evoked activity. In combination, clonidine with either DPDPE or morphine demonstrated a synergistic suppression. No similar synergistic effect was seen when DAGO and clonidine were combined. Our data might suggest that although both mu and delta receptor agonists are antinociceptive, the final common pathways by which they suppress WDR neuron activity are different. The mu-selective agonist DAGO does not seem to synergize with a noradrenergic system.

Supported by NIH Grant NS-09871

341.12

THE ROLE OF SEROTONIN (5-HT) AND NOREPINEPHRINE (NE) IN THE ANALGESIC ACTION OF BETA-ENDORPHIN IN THE SPINAL CORD. T.Crisp, J.L. Stafinsky* and M. Uram*, Dept. Pharmacol., Northeastern Ohio Univ. Coll. of Med., Rootstown, OH 44272 and Dept. Anesthesiology, Western Reserve Care System, Youngstown, OH 44512.

Beta-endorphin (B-E) is an endogenous opioid peptide with antinociceptive properties (Tseng, 1985; Yaksh and Henry, 1978). In the present study, male Sprague-Dawley rats were cannulated with indwelling PE-10 catheters for intrathecal (i.t.) injections. Different doses of B-E (0.1, 1 or 10 nmol) were administered i.t. and the ability of the opioid to inhibit tail-flick latency (TFL) was tested. Spinal B-E dose-dependently elevated TFL, and the 1 nmole dose produced a strong antinociceptive response 30 min post-injection.

In order to assess the local spinal involvement of 5-HT and NE in B-E-induced spinal analgesia, serotonergic or noradrenergic receptor antagonists were administered i.t. and tested against the opioid. The various receptor selective 5-HT blockers (spiroxatrine, ritanserin or ICS 205-930) and the alpha$_2$ antagonist yohimbine reversed the spinal action of B-E. The alpha$_1$ antagonist WB-4101 failed to alter B-E-induced analgesia. These data imply a local spinal interaction between B-E, 5-HT and NE. Supported by Biomed. Res. Grant #3215

341.13

DOES INTRATHECAL SEROTONIN MIMIC THAT RELEASED ENDOGENOUSLY? D.J. Smith, J. Perrotti*, T. Crisp, and D.L. Smith*. Dept. of Anes., WVU, Morgantown, WV 26505.

Serotonin (5HT) is released from spinopetal, pain-inhibitory nerves. Large doses (μmoles) may also be administered intrathecally (i.t.) to induce analgesia (i.e. elevate the latency of the tail-flick (TFL) reflex). In 8 of 14 rats i.t. 5HT (1 μmole) increased TFL 2 S.D. above their individual mean pre-drug values within 10 m. However, it may be questioned if i.t. 5HT mimics the action of neuronally released transmitter. The dose of methysergide required to inhibit the action of i.t. 5HT is much smaller than that needed for 5HT released spinally in response to neuronal activation by i.t. morphine (ID 50 = 0.015 vs. 4.8 mg/kg, s.c. respectively). Also, i.t. 5HT causes other phenomena not observed following the release of endogenous 5HT. These include plaintive vocalization, tremors and reduced blood flow to the eye. Interestingly, these effects, as well as analgesia, may also be observed in rats whose spinal cannula are found to be malpositioned outside of the dura near the lumbar enlargement. In 6 of 11 extradurally cannulated rats, unresponsive to morphine (10 nmole), responses to 5HT were observed. These data raise the concern that some of the actions of i.t. 5HT may be peripheral to the cord (eg. changes in cord blood flow) with secondary influences on nociceptive behavior. WVU Med Corp & NIH 2507RR05433-26.

341.14

AMITRIPYTLINE AND SPINAL ANTINOCICEPTIVE MECHANISMS. G.M. Williams, D.J. Smith, R.H. Docherty*, A.J. Azzaro, L.M. Brown, D.L. Smith* and F. Perrotti*. Dept. of Anes., WVU, Morgantown, WV 26505.

The tricyclic antidepressant amitriptyline (AMT) is used for chronic pain and enhances narcotic action. This study was to determine if spinal mechanisms are involved. Rats were treated with either AMT (10 mg/kg, i.p.) or saline for 21 days. On days, 1, 10 and 17, immediately following AMT, a decrease in latency of the heat-induced nociceptive tail-flick reflex (TFL) occurred, but disappeared within 2 h. Afterwards, the response to intrathecal (i.t.) morphine was enhanced in AMT rats. These effects could result from an AMT-induced inhibition of 5HT reuptake; 1) the transient decrease, a result of the amines action to increase spinal motor neuron activity and 2) the enhanced morphine effect, related to an intensification of its 5HT component. At day 17, hot-plate reaction times did not differ in AMT and control rats nor was the hot plate-induced elevation of subsequently measured TFL changed (HP then TFL, repeated 4x, 10m between pairs). One day following AMT (d 22), baselines remained unchanged from control. Additionally, there was no apparent difference in the potency of i.t. 5HT, nor was there any alteration in the terminal autoreceptor that modulates 5HT release. Thus, AMT does not appear to directly intensify spinal antinociceptive processes.

341.15

DIFFERENTIAL ACTION OF AMITRIPTYLINE ON NEURONS IN THE TRIGEMINAL NUCLEUS. G.H. Fromm and T. Kondo*. Dept. of Neurology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Amitriptyline (AMI) is widely used for the treatment of a variety of painful conditions. This analgesic effect has been corroborated by several controlled studies and appears to be unrelated to AMI's antidepressant effect. However, the exact mechanisms of action of AMI has not been established.

We have investigated the effect of AMI on single neurons in the trigeminal nucleus caudalis of cats anesthetized with alpha-chloralose. The i.v. administration of 1.0-4.0 mg/kg AMI markedly facilitated the periventricular inhibition of nociceptive specific and wide dynamic range neurons. Segmental inhibition was facilitated to a lesser degree. On the other hand, AMI had a mild depressant effect on the periventricular and segmental inhibition of low threshold mechanoceptive neurons.

Our experiments suggest that AMI exerts its analgesic effect by enhancing the action of endogenous inhibitory mechanisms impinging on nociceptive specific and wide dynamic range neurons, especially the inhibition descending from the periventricular gray matter. Such an effect would account for the synergistic action of AMI and opiates that has been reported in both patients and experimental animals.

341.16

COCAINE SUPPRESSION OF MEDIAL THALAMIC NOCICEPTIVE RESPONSES IN THE RAT. C.R. Belczynski Jr., A. Pertovaara, T.J. Morrow and K.L. Casey. VA Medical Center and Depts. of Neurology, Physiology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48105.

Recent behavioral experiments in our laboratory have demonstrated that cocaine (25 mg/Kg, i.p.) is a rapid-onset, non-opiate analgesic in the rat. The effect has been documented using the hot-plate and the formalin tests, and appears to be independent of cardiovascular or local anaesthetic actions of the compound. Analgesic doses of cocaine do not suppress spinal nociceptive reflexes although we have shown that at supraspinal levels, cocaine simultaneously enhances spontaneous activity but reduces noxious-evoked activity of caudally and rostrally projecting medial medullary reticular neurons over a time course paralleling the behavioral analgesia. In the present study, we wished to determine whether cocaine would selectively alter nociceptively-evoked responses of neurons in the medial thalamus without affecting the mechanoreceptive responses of neurons in the lateral ventrobasal complex. Moreover, we investigated whether any observed effects were the result of thalamic or subthalamic mechanisms. Extracellular single cell recording of thalamic neurons in the anaesthetized rat demonstrated that behaviorally analgesic doses of cocaine suppress nociceptively-evoked neuronal activity in midline and intralaminar nuclei. Neuronal evoked responses to central stimulation of the medial bulboreticular formation were also suppressed. Mechanoreceptive responses of neurons in the lateral ventrobasal complex to innocuous tactile stimuli were not suppressed after cocaine administration. Unlike neurons in the medial reticular formation, there were no significant changes in levels of spontaneous activity. The effect of cocaine on neuronal responsiveness was not reversible with the opiate antagonist naloxone. The results suggest that cocaine's analgesic effect is mediated at least in part via a direct and selective suppression of medial thalamic nociceptive responses.

This work was supported by a grant from the Veterans Administration.

341.17

SEROTONIN (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) IN THE BRAINSTEM OF NORMAL AND ARTHRITIC RATS. F.F. Matos*, J.D. Levine* and A.I. Basbaum. Depts. of Anatomy and Medicine, Univ. California, San Francisco, CA 94143.

Previous studies have reported that forebrain, brainstem and spinal cord levels of 5-HT and 5-HIAA are increased in an animal model of chronic pain, the polyarthritic rat (Sofia and Vassar, Arch. Int. Pharm., 211:1974; Weil-Fugazza et al, Brain Res., 175: 1979). We have attempted to identify the source of the increased 5-HT and 5-HIAA, by punch-sampling different brainstem areas. Rats were made polyarthritic by tail injection of Mycobacterium butyricum in mineral oil. Three weeks later the rats were killed by decapitation and the brain rapidly removed and frozen. Only those rats with significant bilateral inflammation were used. 350μ serial crysostat sections through the brainstem were cut; from these 300μ diameter punches were collected, grouped as necessary, and homogenized in $HClO_4$. 5-HT and 5-HIAA levels per mg protein were determined by HPLC with electrochemical detection.

Although the changes previously observed in the spinal cord of the arthritic rat probably reflect activity in 5-HT groups of the rostral ventral medulla, we found no changes in 5-HT or 5-HIAA levels in the nucleus raphé magnus, pallidus or obscurus. The variability in these regions, however, was high. There was a significant increase (approximately twofold) in the level of both compounds in the midbrain dorsal raphé. The levels in various raphé target regions in the brainstem, including the lateral and dorsal periaqueductal gray and the lateral and ventrolateral medulla were unchanged. The increase of 5-HT and 5-HIAA in the dorsal raphé is consistent with the increased levels of 5-HT that have been reported in the forebrain of arthritic rats. These data indicate that changes in 5-HT synthesis in arthritic rats are not only associated with increased activity in the descending serotonergic modulation that arises from 5-HT neurons of the rostral medulla.

Supported by NS14627 and NS21445.

341.18

QUANTITATIVE STUDIES OF BULBOSPINAL SEROTONERGIC NEURONS APPOSED BY ENKEPHALIN FIBERS. W. Wu*, M. Wessendorf and R. Elde. Dept Cell Biol and Neuroanat, Univ Minnesota, Minneapolis, MN 55455

It has been suggested that met-enkephalinergic (met-enk) fibers synapsing onto bulbospinal serotonergic (5-HT) neurons are involved in the descending control of nociception. However, it has been unclear what proportion of descending 5-HT neurons might be involved in such a circuit. It was decided to determine the proportion of descending 5-HT neurons that are apposed by enkephalinergic processes.

Bulbospinal neurons were labeled by injecting the retrograde tracer Fluoro-Gold into the lumbar spinal cord of rats. Serotonin levels were increased by treatment with tryptophan and tranylcypromine prior to sacrifice. Ten μm sections of brainstems were cut and stained for 5-HT and met-enk using 2-color immunofluorescence.

In 2 rats in which the entire cross-sectional area of the lumbar spinal cord was filled with Fluoro-Gold, 3041 descending neurons were sampled in sections of the ventral medial medulla. Out of 677 5-HT neurons observed, 661 (97.6%) were retrogradely labeled with Fluoro-Gold. All of these cells were observed to have met-enk immunoreactive varicosities apposed to them. In an additional 2 rats, we evaluated 105 bulbospinal 5-HT neurons labeled after injections of Fluoro-Gold limited to the dorsal portion of the lumbar spinal cord. Again, all of these cells were apposed by met-Enk varicosites.

These experiments suggest that enkephalinergic afferents may control the activity of bulbospinal serotonergic neurons. In addition, they suggest that virtually all 5-HT neurons in this region project spinally.

These studies were supported by NS22665, DA02148, and IncStar.

341.19

ANTINOCICEPTIVE ACTIONS OF ALPHA-2 ADRENERGIC AGONISTS IN ROSTROVENTROMEDIAL MEDULLA OF THE RAT MAY BE MEDIATED BY A SELECTIVE ACTION ON A SINGLE POPULATION OF PUTATIVE NOCICEPTIVE MODULATORY NEURONS. C.M.Haws*, M.M.Heinricher and H.L. Fields. Depts of Neurol. and Physiol., Univ. CA San Francisco, San Francisco, CA 94143

Two classes of putative nociceptive modulatory neurons have been identified in the RVM of the barbiturate-anesthetized rat; the on-cell shows an increase and the off-cell a decrease in activity just prior to a tail-flick withdrawal response (TF) from noxious heat. The ability of the alpha-2 adrenergic agonist clonidine to produce antinociception following microinjection into the RVM was investigated, and a possible mechanism for this action examined using single unit recording and iontophoretic techniques.

Microinjection of clonidine (1.0-7.5 ug) into RVM produces a dose-related inhibition of the TF (10 sec cut-off) reversible by the alpha-2 receptor antagonist yohimbine but not by the alpha-1 receptor antagonist WB4101.

Iontophoretic application of clonidine produces a yohimbine-reversible suppression of the TF-related on-cell burst. Clonidine has no effect on the off-cell pause.

These results indicate that the antinociceptive effect of clonidine microinjected into the RVM is mediated by a selective action on one population of putative nociceptive-modulatory neurons, on-cells. This would be consistent with a facilitatory effect of on-cells on nociceptive transmission at the spinal level.

Supported by PHS grant NS21445 and the Migraine Foundation.

341.20

DESCENDING MODULATION OF NOCICEPTION FROM THE A5 CELL GROUP A. Burnett and G.F. Gebhart. Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa 52242.

Specific brain regions supplying the spinal cord with monoaminergic innervation important to antinociception and descending inhibition have been the focus of extensive investigation. The well characterized locus coeruleus-subcoeruleus complex (A6 cell group) and the region of the superior olivary nucleus (A5 cell group) are primarily responsible for the noradrenergic innervation of the spinal cord. The present studies, conducted in rats lightly anesthetized with pentobarbital, quantitatively characterized the role of the A5 cell group in descending modulation of the spinal nociceptive tail-flick reflex. Inhibition of the tail-flick reflex was observed at low intensities of stimulation (100 Hz, 100 μs cathodal pulses) near the A5 cell group, associated with significant increases in blood pressure. Glutamate microinjections (100 mM, 0.5 ul) into the same sites as stimulation also inhibited the tail-flick reflex, but generally lowered blood pressure. Intensity-, frequency-, and pulse width-dependent effects of electrical stimulation and dose-dependent effects of chemical stimulation were studied. The possible contribution of the A5 cell group as an interface between cardiovascular and pain sensing/modulating systems was evaluated. Supported by DA02879.

PAIN MODULATION: CENTRAL PATHWAYS II

342.1

ROLE OF ANTERIOR PRETECTAL NUCLEUS (APT) IN SOMATOSENSORY CORTICAL DESCENDING MODULATION OF JAW-OPENING REFLEX (JOR) IN RATS. L.-D. Lin, C.Y. Chiang*, J.O. Dostrovsky and B.J. Sessle. Dept. Physiology, Fac. Medicine and Fac. Dentistry, Univ. of Toronto, M5S 1A8, Canada.

Since we previously noted that stimulation of the somatosensory cortex (CX) or APT produces inhibitory effects on the JOR and since the APT receives ipsilateral descending projections from CX (Wise & Jones, J. comp. Neurol., 175,129,1977), we examined whether the APT is involved in the CX descending modulation of the JOR. The digastric JOR was elicited in 60 chloralose/urethane-anaesthetized rats by test stimulation (0.1-0.3ms, 0.5Hz) of the maxillary skin or lower incisor tooth. Conditioning stimulation (8 pulses, 400Hz, 0.2ms) of CX or APT induced JOR inhibition of similar amplitude (average 60% reduction) and time course (average onset 25ms, peak at 50ms, duration 200ms). Prior lesioning of APT by ibotenic acid markedly diminished the CX-induced inhibition. Glutamate (0.2M, 200-600nl) injected into APT and adjacent ventral regions also inhibited the JOR, but electrical or glutamate-induced inhibition from APT was less than the JOR inhibition induced from caudal periaqueductal gray (PAG). Lidocaine-produced block of caudal PAG function reversibly reduced by 50% the APT-induced inhibition, whereas block of the rostral PAG had no clear effect on the inhibition. These findings suggest that the APT may at least partially mediate the CX descending modulation of JOR and that the caudal PAG may be involved in part in mediating this effect.

342.2

STIMULATION-PRODUCED ANALGESIA FROM THE NUCLEUS TRACTUS SOLITARIUS IN THE UNANESTHETIZED RAT. J.-H. Sohn, A. M. Lohof, M. M. Morgan and J. C. Liebeskind. Department of Psychology, UCLA, Los Angeles, CA 90024-1563.

Electrical or chemical activation of the commissural region of the nucleus tractus solitarius can produce analgesia in the pentobarbital anesthetized rat (Lewis et al., '87; Morgan et al.'87; Randich & Aicher, '88). Implantation of chronic indwelling electrodes into the NTS is made difficult by the caudal location of this nucleus. The present study describes a technique for implantation of chronic indwelling electrodes into the NTS, and evaluates stimulation-produced analgesia (SPA) in the conscious rat. Rats were anesthetized and placed in a stereotaxic frame. An incision was made and the trapezius muscles gently retracted from the medial occipital bone. The atlanto-occipital membrane membrane was punctured and the ventral medial part of the occipital bone removed. A tear was made in the arachnoid and a twisted bipolar electrode positioned over the obex. The electrode was bent in two places to conform to the shape of the skull. The electrode was lowered 0.8 mm into the caudal NTS and affixed to screws in the top of the skull with dental cement. One week after surgery, pain sensitivity was assessed with the tail-flick test. Brain stimulation consisted of 50 Hz monophasic pulses (0.4 ms) preceding by 20 s and remaining on during the tail-flick test. Eight of the 9 stimulation sites within the NTS supported SPA, at a mean current of 238 μA. None of the four stimulation sites located outside the NTS supported SPA. Analgesia occurred in the absence of aversive or motor reactions. These results indicate that SPA from the NTS can be obtained in unanesthetized animals and does not appear to be secondary to overt aversive or motor effects. (NIH grant NS07628)

342.3

HYPOTHALAMIC PVN STIMULATION PRODUCES ANALGESIA NOT MEDIATED BY VASOPRESSIN OR OPIOIDS. R. Yirmiya, S. Ben-Eliyahu*, Y. Shavit, P. Marek* and J.C. Liebeskind. Department of Psychology, University of California, Los Angeles, CA 90024.

The analgesic effect of hypothalamic paraventricular nucleus (PVN) stimulation and the involvement of vasopressin and opioid peptides in this process were studied in vasopressin-deficient (Brattleboro) and Long Evans rats. Rats were chronically implanted with a monopolar stimulating electrode in the PVN. Ten days after surgery, the animals were lightly anesthetized and PVN stimulation-produced analgesia (SPA) threshold was determined by the tail-flick test. Half the animals were then injected with naloxone (10 mg/kg) and half with saline. SPA threshold was determined again 20 min later. The same procedure was repeated on the following day, except that the drug assignment was reversed. PVN SPA threshold did not significantly differ between Brattleboro and Long Evans rats. Threshold was stable over the two sessions and was not significantly changed by naloxone. The result indicate that the analgesic effects of PVN stimulation are not mediated by either vasopressin or opioid peptides. Supported by NIH grant NS 07628.

342.4

ANALGESIA PRODUCED BY INJECTION OF LIDOCAINE INTO THE ANTERIOR CINGULUM. A.L. Vaccarino and R. Melzack* Dept. of Psychology, McGill University, Montreal, P.Q., Canada, H3A 1B1.

Several investigators have demonstrated thalamic involvement in pain perception. The cingulum, because of its intimate relationship between the thalamus and other limbic and cortical structures, may provide a possible mechanism by which pain signals can be processed at higher brain regions involved in emotion, cognition and motivation. In the present study, the role of the cingulum in both tests of phasic and tonic pain are examined using injections of a local anaesthetic into the cingulum region.

Male Long-Evans rats were stereotaxically implanted with unilateral cannulae aimed at the anterior portion of the cingulum. Seven to ten days following surgery the rats were infused, via an inner cannula, with 1 µl of 2% lidocaine in saline or saline alone over a 2 minute period and tested for analgesia in either the formalin or foot-flick test.

Infusions of lidocaine produced a significant reduction in formalin pain scores, but had no effect on foot-flick latencies. Furthermore, contralateral lidocaine appeared to have a greater analgesic effect than ipsilateral lidocaine. The possible additive effects of bilateral lidocaine injection are currently under investigation.

These data strongly suggest cingulum involvement in pain perception and support previous findings dissociating phasic and tonic pain mechanisms. Studies examining the involvement of other brain regions anatomically associated with the cingulum are currently in progress. Supported by NSERC grant A7891

342.5

EFFECTS OF DORSAL RAPHE, HABENULA AND EXTERNAL ELECTRICAL STIMULATIONS ON SEPTAL NUCLEUS NEURONS IN THE RAT. W.-Q. Dong, M. Skolnick and N. Dafny. The Univ. of Texas Health Sci. Center at Houston, 77225.

It was recently proposed that most CNS structures which participate in a pain modulating mechanism will respond to noxious input, and when stimulated will induce analgesia (SPA). The mechanism underlying this effect has remained an open question. Several reports have suggested that electrical stimulation applied to the septal nucleus will elicit analgesia. In order to test this hypothesis, we investigated the effects of focal stimulation of the dorsal raphe (DR) and habenula (Hab) nuclei, and pinnael (ear) electrical stimulations (PES) on the septal nucleus of the rat. Twenty-five urethane-anesthetized Sprague-Dawley rats were used. Stainless steel bipolar stimulating electrodes were placed within the DR and Hab, and external electrical stimulating electrodes were inserted into the pinnae of each animal. Micropipettes (2M NaCl) were used as recording electrodes. It was observed that the septal nucleus cells exhibit 2 types of responses to noxious stimulation (tail pinch): "nociceptive-on" and "nociceptive-off". In addition, PES was more effective as anti-nociceptive than DR and Hab stimulation."
Supported by American Health Services Corporation, Inc.

342.6

SEROTONIN INVOLVEMENT IN ANALGESIA INDUCED BY TRANSCRANIAL ELECTROSTIMULATION. L. Hudson-Howard*, R. Warner*, C. Johnston* and M. Skolnick (Spon: B. Ho) Neurophys. Res. Cent., Univ. of Texas Health Sci. Cent., Houston, TX 77030

External transcranial electrostimulation (TE) consisting of charge-balanced, rectangular, constant current (10-15 µA) low frequency pulses has been shown to produce endorphinergic analgesia in rats. This report summarizes investigation of serotonergic involvement in TE-induced analgesia. Subjects were 200g, male, Sprague-Dawley rats naive to TE but conditioned to handling and restraint.

P-chlorophenylalanine (pCPA) was injected to block the biosynthesis of serotonin and subjects were then tested for analgesia. The rats were restrained and stimulated. Analgesia was assessed using a pressure tail flick test. Pressure (½ in. from the tip) was exerted by a metal wedge mounted on a pneumatically driven syringe plunger. Maximum pressure tolerated by the rat was read as the rat made the first coordinated motor response to move its tail. This reading was averaged over four trials to produce the tolerated peak pressure (TPP). The difference in mean TPP before and after TE was taken as a measure of analgesia. The experimenter was blind to the conditions of the experiment.

This 2x2 factorial design demonstrated a 5HT dependent analgesic effect: pCPA blocked the TE-induced analgesia. The TE treated, saline injected group shown a significant increase in mean TTP vs. all other groups--TE-pCPA, Sham-pCPA, sham-saline: N=62 $p<.01$ Tukey HSD.

342.7

ENKEPHALINASE INHIBITOR THIORPHAN POTENTIATES ANALGESIC EFFECT OF TRANSCRANIAL ELECTROSTIMULATION (TCES). J.R. Lake*, R.F. Hamilton*, M. Skolnick, and D.H. Malin (Spon: I.J. Butler) Univ. of Houston-Clear Lake, Houston, Tx 77058; Univ. of Texas Grad. School of Biomedical Sci., Houston, Texas 77030.

Naloxone-reversible analgesia is induced by bilateral ultralow current stimulation of low impedance points on the ear (TCES). The present study tested the ability of thiorphan to potentiate analgesic effects of TCES by amplifying the effects of enkephalin release. Rats (n-20) were cannulated in the 3rd ventricle and implanted with electrodes through the apex of the antihelix. Rats were pretested for latency in the 50°C wet tail flick test. Ten rats then received 30 min. of TCES (10 Hz, 10 µA, charge-balanced rectangular pulses). Ten rats received 30 min. of "sham stimulation". Half of each group received 250 µg thiorphan icv in 100 µl 3% ethanol (5 µl/min). The other half received injection vehicle alone. All rats were then retested. Analgesia scores were increases in latency (sec.s) from pretest, as shown below.

Effects of TCES and Thiorphan on Analgesia Scores M ± SEM

	TCES	SHAM STIMULATION
THIORPHAN	**3.4 ± 0.5***	**1.9 ± 0.4**
VEHICLE	**1.0 ± 0.3**	**0.1 ± 0.3**

ANOVA reveals both drug and TCES effects, $p<.01$. The thiorphan + TCES group was different, $p<.05$, from all other groups. TCES analgesia may thus be mediated by enkephalin release and intensified by enkephalinase inhibition.

342.8

MODULATION OF CORTICAL SENSORY EVOKED POTENTIALS (SEP) BY STIMULATION IN NUCLEUS RAPHE MAGNUS (NRM) IN RATS. K.A. Follett and G.F. Gebhart. Depts. of Neurosurgery and Pharmacology, Univ. of Iowa, Iowa City, Iowa, 52242.

Midbrain electrical stimulation has been reported to reduce the amplitude of SEPs. Antinociceptive effects of midbrain stimulation are mediated via nuclei of the ventral medulla, including NRM. The purpose of this study was to determine whether SEPs could also be modulated by stimulation of NRM.

A condition-test (CT) paradigm was used. Rats were anesthetized with Nembutal, N_2O and O_2, and paralyzed with pancuronium. The SEPs evoked by test stimuli (6 mA, 0.5 msec, bipolar needle electrodes) delivered to the hindpaw were recorded epidurally. Conditioning stimuli (CS) (10 pulses, 0.1 msec each) were delivered to the NRM through a monopolar electrode positioned stereotaxically.

An adequate CS preceding the test stimulus consistently reduced the amplitude of the SEP. Threshold for the effect was as low as 25 µA with near complete abolition of the SEP by CS intensities of 50-100 µA. No latency changes were observed consistently. High CS pulse frequency (400 Hz) was more effective than lower frequencies. SEP reduction was most pronounced with CT intervals of <20-50 msec.

The attenuation of SEPs by NRM stimulation may occur through depression of cortical responsiveness, blocking or occlusion at a cortical level, or by inhibition of afferent pathways at subcortical levels.

342.9

LIMBIC INFLUENCE ON INTRACELLULAR ACTIVITY OF VENTRAL MEDULLARY NEURONS. D. Borsook, S. Potrebic*, A. Strassman, R. Maciewicz. Pain Physiology Laboratory, Mass General Hospital, Boston MA 02114.

Descending projections from several limbic regions terminate in the midbrain periaqueductal gray (PAG) and nuclei of the ventromedial medulla (VM). These pathways provide a potential substrate for the analgesic effects of limbic forebrain stimulation. The present study investigated the effects of limbic stimulation on intracellular responses of VM cells in the cat. Stimulating electrodes were placed in the central amygdaloid nucleus (ACE), the anterior hypothalamus (AH), the bed nucleus of the stria terminalis (BNST), the septum, and the PAG. The final electrode placements were selected by minimizing the stimulation intensity required to suppress the tooth pulp-evoked jaw opening reflex. The majority of neurons in VM responded to PAG stimulation with a short latency, monosynaptic EPSP. Single shock or short train stimulation of ACE, the BNST, and AH also evoked mono- or oligo- synaptic EPSPs in many of these same neurons. Cells excited by ACE or BNST were intracellularly stained with HRP. Labeled cells were large multipolar or medium fusiform neurons located in raphe magnus or the adjacent magnocellular reticular formation. These results are evidence that analgesia associated with stimulation of limbic structures may be mediated by activation of antinociceptive pathways involving cells in the ventromedial medulla.

342.10

EFFECTS OF SINGLE AND CONJOINT MET-ENKEPHALIN MICRO-INJECTIONS IN RAT CENTRAL GRAY (PAG) AND NUCLEUS RETICULARIS PARAGIGANTOCELLULARIS (PGC) ON ACTIVITY OF BULBAR RAPHE (RM) AND RAPHE-SPINAL (RMS) NEURONS. J.P. Rosenfeld and L.Y. Xia*. Cresap Neuroscience Lab., Northwestern University, Evanston, IL 60208

As of submission date, 65 neurons histologically identified as being within the RM nucleus of 38 rats were studied. The rats were anaesthetized under 65 mg/kg Pentabarbitol for surgical preparation, and then maintained in light anaesthesia (with 20% initial dose per hour, IM) adequate to allow tail flick reflex in > 70% of the cases. Wound margins were locall anaesthetized with lidocaine. Rats were maintained at 36-38°C anal temperature. Neurons showing spontaneous activity were tested for spinal projection with bipolar silver ball antidromic stimulation between left T9 and right T10 dorsal surface of spinal cord, followed with collision testing. Only 8 cells satisfied the RMS criteria. Spontaneous activity of 3% of the RM cells was augmented by light touch, 2% were inhibited and most (95%) showed no response. Noxious heat enhanced spontaneous RM firing in 56% of the neurons, inhibited it in 37%, and had no effect in 3% of cases. Noxious pinch had similar effects. All body surfaces from nose to tail were tested. Effects on nose and tail were usually parallel, however in 4% of the cells, nose and tail stimulation produced different effects. (Thus 56% + 37% + 3% + 4% = 100%). Considering all 57 RM cells, single and conjoint Met-Enkephalin injections of PAG (2 min., 200 nl, 10 ug) and PGC (1 min., 100nl, 5 ug) produced enhancement of spontaneous activity (>50%) in about 1/3 of the tests and depression in 2/3 of the tests. The most significant finding was that the effect of enkephalin injection could be predicted largely by the effect of noxious stimulation: In those RM cells whose firing levels were enhanced by noxious stimulation, 100% (17 of 17) were depressed by PAG injection, 100% (18 of 18) were depressed by PGC injection, and 97% (34 of 35) were depressed by conjoint injection. In those RM neurons whose firing rates were depressed by noxious stimulation, 100% (8 of 8) of the rates were enhanced by PAG injection, 90% (9 of 10) were enhanced by PGC injection and 100% (16 of 16) were enhanced by conjoint microinjection. These patterns obtained for RMS as well as RM neurons. (Supported by NIH grant DE 07905.)

342.11

ANTINOCICEPTION INDUCED BY MICROINJECTION OF CARBACHOL IN THE VENTRAL ROSTRAL MEDULLA IS NOT MEDIATED BY ENKEPHALINERGIC NEURONS. M.A. McCartney and H.K. Proudfit. Dept. Pharmacology, Univ. Illinois at Chicago, Chicago, IL 60612.

The ventral rostral medulla (VRM) contains cholinergic terminals, muscarinic receptors, and microinjection of the cholinergic agonist, carbachol into the n. raphe magnus (NRM) produces antinociception. However, the effects of carbachol microinjected into the n. reticularis gigantocellularis pars alpha (NGCA), n. reticularis gigantocellularis (NGC), and n. reticularis paragigantocellularis (NRPG) have not been evaluated. Therefore, studies were done to determine whether microinjection of carbachol into these sites produces antinociception. In addition, the role of endogenous enkephalins in mediating carbachol-induced antinociception was examined.

Microinjection of carbachol (2.5 ug) into the NRM, NGC, NGCA, but not NRPG, produced antinociception. However, this antinociception does not appear to be mediated by enkephalins since naloxone, administered either systemically or intrathecally, failed to reverse the carbachol-induced antinociception. This work was supported by USPHS Grant DA 03980.

342.12

BEHAVIORALLY DERIVED REFRACTORY PERIOD ESTIMATES OF THE SUBSTRATES FOR ANALGESIA DERIVED FROM STIMULATION OF THE DORSAL AND VENTRAL PAG. Susan Schenk and Kim Pollard-Smith*. Texas A&M University, Dept. Psychology, College Station, TX 77843

Psychophysical methods were used to obtain refractory period estimates of the directly stimulated substrate for the analgesic effects of periaqueductal gray (PAG) stimulation. Trains of stimulation pulses (10 sec. train lengths, 0.1 msec monophasic constant current cathodal pulses) were delivered to the dorsal or ventral PAG of restrained rats. Immediately following the stimulation, the caudal 2.5 - 3.0 mm of the rat's tail was immersed in heated water (52 -54°C) and latency to tail flick was measured. Frequency threshold for analgesia was determined as the frequency of stimulation that resulted in a tail flick latency longer than 6 sec. Pairs of stimulation pulses were also delivered at intra-pulse pair intervals of 1.0 - 10.0 msec. Frequency thresholds for analgesia under this stimulation condition were compared to the threshold when only single pulses were delivered. Results indicated that the effectiveness of the paired pulse stimulation increased gradually as pulse-pair interval was increased from 1.5-7.5 msec. for both the dorsal and ventral sites. These data suggest that the analgesic properties of stimulation derived from dorsal or ventral PAG sites rely on the direct activation of similar caliber neurons.

342.13

GABA-IMMUNOREACTIVE SYNAPTIC CONTACTS ONTO PROJECTION NEURONS OF THE PERIAQUEDUCTAL GRAY MATTER AND THE NUCLEUS RAPHE MAGNUS. D. B. Reichling, H.-J. Cho* and A. I. Basbaum. Depts. Anatomy and Physiology, Univ. of California, San Franicisco, CA 94143.

GABA antagonists microinjected into the midbrain periaqueductal gray matter (PAG) or into the medullary nucleus raphe magnus (NRM) inhibit the nocifensive tail-flick reflex. Conceivably, this effect is due to the blockade of a tonic GABAergic inhibition of projection neurons from the PAG to the NRM, or from the NRM to the spinal cord. This report describes anatomical evidence for GABAergic modulation of midbrain/medullary antinociceptive pathways.

For PAG studies, WGA-apoHRP conjugated to colloidal gold was microinjected into the NRM of rats, 5 days prior to perfusion. Silver-enhanced, Vibratome sections of the PAG were embedded, thin-sectioned, and immunostained (on grids) with an anti-GABA antibody. Approximately 40% of terminals in the PAG are GABA-immunoreactive (IR). These contain round vesicles, and form symmetrical synaptic contacts with dendrites and cell bodies, but not with axons. Approximately 50% of GABA-IR profiles contain dense-core vesicles. About half of synaptic contacts onto retrogradely labelled cell bodies are GABA-IR; none of these contain dense-cored vesicles.

For NRM studies, WGA-HRP was injected into the spinal cord, and an electrolytic lesion was made in the PAG. Approximately 48% of terminals in the NRM are GABA-IR; these contain round vesicles and are presynaptic to dendrites and cell bodies. About 3% of GABA-IR terminals made asymmetrical contacts and contained flat vesicles. Degenerating PAG terminals and GABA-IR terminals converge onto raphe-spinal projection neurons. These data indicate there are multiple sites of GABAergic modulation of descending antinociceptive control. Supported by NS14627 and NS21445.

342.14

THE RELATIONSHIP OF PERIAQUEDUCTAL GRAY PROJECTIONS TO BULBOSPINAL NEURONS: A COMBINED FLUOROGOLD-PHA-L ANALYSIS. A.J. Beitz, M.A. Mullett* and N. Brandt*, Dept. of Vet. Biol., Univ. of Minnesota, St. Paul, MN 55108

The present study was designed to determine the location of brain stem neurons that receive direct input from the periaqueductal gray (PAG) and project to the spinal cord. Ten adult Sprague-Dawley rats received multiple injections of 4% Fluoro-gold into the lower cervical spinal cord and a single iontophoretic injection of phaseolus vulgaris leucoagglutinin (PHA-L) into the midbrain PAG. Following fixation, the brains were sectioned and PHA-L labeled fibers were visualized using immunofluorescence. The mean number of retrogradely labeled neurons (per 0.04 mm^2 area per nucleus per section) that were contacted by PHA-L labeled fibers were quantitated. The nuclei containing the greatest number of spinobulbar neurons, which appeared to be contacted directly by PHA-L labeled PAG projection neurons, were the gigantocellular reticular nucleus pars alpha, nucleus subcoeruleus, lateral paragigantocellular nucleus, pedunculopontine tegmental nucleus, oral pontine reticular nucleus, ventral gigantocellular reticular nucleus, ventrolateral tegmental nucleus, raphe magnus and rostroventrolateral reticular nucleus. These results suggest that several brainstem nuclei may relay PAG input to the spinal cord and quantitatively the raphe magnus does not appear to be the most significant. Supported by NSF grant BNS-8607520 and NIH grants NS 19208, DE 06682 and DA 04090.

342.15

TRIGEMINAL AND PAG MODULATION OF OPIOID PEPTIDE GENE EXPRESSION IN NUCLEUS CAUDALIS. T. Nishimori*, M. Moskowitz, D. Borsook, R. Maciewicz and G. Uhl (SPON: R.W. Kunkl). G.NSci. Unit NIDA, Depts. of Neurol. & NSci., JHU Med. School, Balto., MD 21224 & MGH, Boston, MA 02114

We have used in situ hybridization to define the expression of the preproenkephalin and preprodynorphin genes in neurons of laminae I and II of the trigeminal nucleus caudalis.

Neurons expressing preprodynorphin were more likely to be found in lamina I and the outer layer of lamina II; neurons expressing preproenkephalin were more uniformly distributed within laminae I and II.

After lesions of the trigeminal ganglion, neuronal expression of preproenkephalin decreases, due to a decline in the number of neurons expressing this gene, while the number of neurons expressing preprodynorphin increases.

0.1mA stimulation of the trigeminal ganglion increases the number of neurons expressing preproenkephalin, while preprodynorphin mRNA expression in the same animals and with electrostimulation of the PAG shows a more complex pattern of changes.

These results support specific patterns of opioid peptide gene regulation by both primary afferents and descending inputs from the PAG, and provide examples of opposite function-related changes in expression of the two principal brain opioid peptide genes.

342.16

THE MECHANISM OF INHIBITION OF SPINOTHALAMIC TRACT (STT) NEURONS BY ELECTRICAL STIMULATION OF PERIAQUEDUCTAL GRAY (PAG). D. Zhang, C.M. Owens and W.D. Willis, Dept. of Anat. & Neurosci. and Marine Biomed. Inst., Univ. of Texas Medical Branch, Galveston, TX 77550.

The activity of STT neurons can be inhibited by PAG stimulation. The present study employs extracellular and intracellular recording and horseradish peroxidase (HRP) injection to investigate the spinal mechanism involved in such inhibition. Ten anesthetized adult monkeys (M. fascicularis) were used. Extracellular recording from 7 STT neurons confirmed that PAG stimulation preferentially inhibited the responses to sural nerve C-fiber volleys regardless of cell location or type. Intracellular recording and injection of HRP into 7 STT neurons revealed a positive relationship between the response of the neurons to C-fiber volleys and the projection of dendrites into laminae I and II. PAG stimulation evoked a hyperpolarizing potential and a concomitant inhibition of discharges in 5 neurons. In 2, the resting membrane potential was altered by current injection. The amplitude of the hyperpolarization was increased with hyperpolarizing current and decreased with depolarizing current, indicating disfacilitation rather than an inhibitory postsynaptic potential. Both neurons had small responses to C-fiber volleys and one of them that was labeled with HRP had no dendrites extending to laminae I or II. (Supported by NIH grants NS 09743 and NS 11255.)

342.17

NEUROCHEMICAL LATERALITY OF THE ANALGESIC EFFECT OF PAG STIMULATION IN THE MOUSE. J. C. Liebeskind, R. Yirmiya and P. Marek* (SPON: J. Lewis). Department of Psychology, University of California, Los Angeles, CA 90024 and Institute of Genetics and Animal Breeding, Polish Academy of Sciences, 05-551 Mrokow, Poland.

The mechanisms of periaqueductal gray (PAG) stimulation-produced analgesia have been widely studied in the rat. In the present study, we compared the involvement of opioids in the analgesic effect of stimulating different regions of the PAG in mice. Swiss-Webster mice were anesthetized with pentobarbital and a monopolar stimulating electrode was stereotaxically positioned in the ventral or the dorsal lateral PAG. Nociception was assessed using the hind paw flick test (paw withdrawal from radiant heat). After determination of SPA current threshold for each paw separately, all animals received naloxone (5 mg/kg, i.p.) and SPA current thresholds were estimated again 20 min later. SPA threshold did not differ between dorsal and ventral parts of the PAG. Naloxone equally attenuated SPA threshold from both areas. The SPA threshold for the paw contralateral to the stimulation site was half that for the ipsilateral paw. Moreover, naloxone attenuation of SPA was 3 times greater for the contralateral than for the ipsilateral paw. Preexposure to analgesic neck scruff pinch almost completely abolished the antinociceptive effect of contralateral PAG stimulation, suggesting the development of functional tolerance. Naloxone administration before pinching attenuated this effect. These results suggest a contralateral organization of the opioid component of the pain inhibitory system in the PAG. Supported by NIH grant NS07628.

342.18

GENETIC MODULATION OF PAG STIMULATION PRODUCED ANTINOCICEPTION IN MICE. P. Marek*, R. Yirmiya, I. Panocka* and J. C. Liebeskind. Institute of Genetics and Animal Breeding, Polish Academy of Sciences, 05-551 Mrokow, Poland, and Department of Psychology, University of California, Los Angeles, CA 90024.

Strain differences in the analgesic effect of morphine, electroacupuncture and stress in mice indicate modulation of the endogenous pain inhibitory system by genetic factors. In the present study the antinociceptive effect of electrical periaqueductal gray (PAG) stimulation and its naloxone reversibility were compared in four genetically different strains/lines of mice: CXBK (deficient in brain opiate receptors), CXBH (rich in brain opiate receptors), LA and HA mice (selectively bred for low and high swim induced analgesia, respectively). Animals were anesthetized with pentobarbital and a monopolar stimulating electrode was stereotaxically positioned in the lateral PAG. Nociception was assessed using the hind paw flick test (paw withdrawal from radiant heat). After determination of SPA current threshold, all animals received naloxone (5 mg/kg, i.p.) and SPA current threshold was estimated again 20 min later. SPA threshold did not differ between CXBK and CXBH mice. However, naloxone caused a three-fold increase of threshold in CXBH mice, but was ineffective in CXBK mice. SPA threshold in HA mice was half that of LA mice. Naloxone significantly increased SPA threshold in HA, but not LA mice.These results indicate that genetic factors importantly affect the neurochemical mediation of endogenous analgesic processes. Supported by NIH grant NS07628 and the Polish Academy of Sciences project CPBP-04.01/6.13.

HIPPOCAMPUS AND AMYGDALA III

343.1

AMYGDALA DIRECTLY INNERVATES BRAINSTEM CATECHOLAMINERGIC CELLS IN THE RAT. T.S. Gray and D.J. Magnuson*. Dept. Anatomy, Stritch School of Medicine, Maywood, IL 60153

The present study used a combined phaseolus vulgaris leucoagluttin lectin anterograde tracer (PHA-L) and immunocytochemistry to determine whether amygdala cells directly innervated tyrosine hydroxylase, PNMT and/or dopamine beta hydroxylase immunoreactive cells within the brainstem. Iontophoretic injections of PHA-L were placed within the central nucleus of the amygdala of anesthetized 150-250g Long-Evans rats. Two weeks later animals were overdosed with sodium pentobarbital and their brains were fixed through vascular perfusion. Amygdaloid terminals were demonstrated by antibodies to PHA-L with avidin-biotin immunocytochemistry using a brown DAB reaction. Catecholaminergic cell bodies were visualized via antibodies to TH, PNMT or DBH using a glucose oxidase-nitro blue tetrazolium reaction. Amygdaloid terminals were distributed within a variety of areas that were immunoreactive to catecholamine markers. For example, amygdaloid terminals appeared to contact tyrosine hydroxylase immunoreactive cells within the substantia nigra, locus coeruleus and the A8 cell group. Amygdaloid terminals also appeared to contact TH and PNMT immunoreactive cell bodies within the nucleus of the solitary tract and the ventrolateral medulla. The results demonstrate that the amygdala can directly influence a number of subpopulations of catecholaminergic neurons within the brainstem. (Supported by NIH NS 20041)

343.2

VIDEO MICROSCOPY OF CULTURED AMYGDALA BRAIN SLICES. D.E. Stevens*, E.W. Kairiss, P.F. Chapman*, and T.H. Brown. (SPON: C.L. Keenan) Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Long-term synaptic potentiation (LTP) was recently discovered to occur in synapses of the acute amygdala brain slice (P.F. Chapman and T.H. Brown, this meeting). To increase the accessability of this complex structure to high-resolution analysis of such synaptic phenomena, we have applied the roller tube culture procedure, which was previously developed (Gahwiler, B.H. *J. Neurosci. Meth.*, 4:329, 1981) for use on hippocampal tissue.

Horizontal slices of amygdala (including adjacent regions of cortex, fundus striati, dorsal endopiriform nucleus, lateral hypothalamus and subiculum) were prepared from the brains of 6-8 day old rat pups previously anesthetized with halothane. The slices were cultured on collagen-coated coverslips and maintained for periods of up to several weeks. Our preliminary studies have focussed on the development of the cultures and the morphological characterization of cultured amygdala neurones. The cytoarchitecture and cellular morphology were examined using video-enhanced contrast, differential-interference contrast (VEC-DIC) microscopy.

By 2 weeks *in vitro* the cultures had flattened into a 1-6 cell layer structure. Low-power (4X-10X)VEC-DIC microscopic observation of the living slices and of cresyl violet stained slices revealed the presence of recognizable amygdaloid nuclear regions as well as cortical cell layers. When viewed at higher power (40X) magnification, individual neuronal somata were readily visualized in the living tissue. Intracellular injections of lucifer yellow revealed several types of neuronal morphologies that were similar to those seen in published golgi material.

We conclude that the cultured amygdala slice preparation holds great potential for combining biophysical and neurophysiological analysis with visualization techniques such as VEC-DIC microscopy and confocal scanning laser microscopy. These combined approaches should expedite our understanding of the biophysics and microphysiology of synaptic transmission in this important but complex temporal lobe structure. *(Supported by AFOSR and Beckman Research Institute)*

343.3

STIMULATION OF THE AMYGDALOID CENTRAL NUCLEUS (ACe) FACILITATES THE NICTITATING MEMBRANE UNCONDITIONED REFLEX (NMUR) IN THE RABBIT. P.J. Whalen and B.S. Kapp. Dept. of Psychology, Univ. of Vermont, Burlington, VT 05405.

The maintenance of facilitation of the NMUR during Pavlovian conditioning appears to involve an associative learning process (Weisz and McInerney, 1987). Since the ACe (a) contributes to the modulation of various reflexes (Gary Bobo and Bonvallet, 1975; Schlor et al., 1984; Pascoe et al., 1987), (b) projects to the brainstem lateral tegmental field which contains the circuitry for the NMUR (Hopkins and Holstege, 1978) and (c) has been implicated in associative learning (Kapp et al., 1986), the present study was conducted to investigate the contribution of the ACe to facilitation of the NMUR.

New Zealand rabbits were prepared with stimulation electrodes (200um tip) in the ACe. Following recovery, the NMUR was elicited over 16 trials. Electrical stimulation of the ACe, at an intensity which influenced the brainstem as indexed by vagal bradycardia, was presented for either 400 or 100msec prior to reflex elicitation for eight of these trials (<100uamps, 100Hz). A significant increase in reflex amplitude (10-24%, ps<.05) was observed on ACe stimulation trials when compared to trials not preceded by stimulation. Stimulation of the ACe in the absence of NMUR elicitation did not induce membrane movement. These results are consistent with the notion that the ACe may contribute to associative facilitation of the NMUR.

343.4

CONNECTIONS OF THE PREFRONTAL CORTEX WITH THE HIPPOCAMPAL FORMATION IN THE CAT AND MACAQUE MONKEY. C. Cavada and F. Reinoso-Suárez. Dept. Morfología, Fac. Medicina, Univ. Autónoma, Madrid, Spain.

Injections of anterograde and retrograde tracers were made in different sectors of the prefrontal cortex (PfC) in cats and macaques, and the resulting labeling in the hippocampal formation was analyzed. Serial sections adjacent to those processed to reveal the transported tracers were stained for Acetyl Cholinesterase (AChE) histochemistry.

Our results indicate that only the dorsolateral sector of macaque PfC projects intensely to the presubiculum. In cat, the ventral PfC projects to the parasubiculum. The terminal labeling in this region is densest in the deep part of layer I, and overlaps with an intensely stained AChE band. In both cat and monkey, the subiculum projects heavily to the ventral PfC. All prefrontal sectors studied are connected with the so called caudomedial lobule in macaque and caudomedial band in cat.

These findings suggest that the connections of the PfC with the hippocampal formation exhibit notable similarities in the two species studied. This argues in favor of the existence of comparable functions of the prefronto-hippocampal loops. However, connectional differences are also present, which may entail functional implications.

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343.5

BASAL FOREBRAIN AFFERENTS, MUSCARINIC RECEPTORS AND PUTATIVE PRESYNAPTIC CHOLINERGIC MARKERS IN THE HIPPOCAMPAL FORMATION AND ENTORHINAL CORTEX OF THE RHESUS MONKEY. K.J. Rhodes, D.L. Rosene, and M.B. Moss. Dept. of Anatomy, Boston Univ. Sch. Med., Boston, MA 02118.

We have compared the distribution of basal forebrain (BF) afferents with muscarinic receptors and putative presynaptic cholinergic markers in the hippocampal formation (HF) and entorhinal cortex (EC). BF afferents to the HF and EC were labeled using anterograde transport of [3H]-labeled amino acids. Muscarinic receptors were labeled with [3H]-pirenzepine (M1) and [3H]-oxotremorine (M2). Acetylcholinesterase (AChE) and [3H]-hemicholinium-3, which binds to high affinity choline uptake sites (HACU), were used as putative presynaptic markers. In the HF the distribution of BF afferents, AChE and HACU showed close correspondence except in stratum moleculare (SM) of the dentate gyrus (DG) where BF afferents and HACU were uniformly distributed but AChE reaction product was most dense in the inner 1/3. Like BF afferents, AChE and HACU were distributed densely in strata oriens and pyramidale of CA1-4 and in the prosubiculum. However, M1 and M2 sites did not follow the distribution of BF afferents, AChE, or HACU sites in several areas. For example, in SM of the DG M1 sites were very dense compared with BF afferents, AChE, M2 and HACU. In the subiculum, M2 sites were very dense compared with BF afferents, AChE, M1 and HACU. In EC, BF afferents, AChE, HACU and M1 sites showed small laminar variations in distribution but M2 sites were dense in layer III, especially in area 28S. In the HF, AChE and HACU are good markers for BF cholinergic afferents except in the inner 1/3 of SM of the DG. In the subiculum and layer III of EC, M2 sites are very dense while other cholinergic markers are not. (Supported by NS19416, AG00001, AG04321 and T32-NS07152)

343.6

DISTRIBUTION OF CORTICAL PROJECTIONS TO THE MONKEY ENTORHINAL CORTEX: AN AUTORADIOGRAPHIC STUDY. R. INSAUSTI AND D.G. AMARAL. The Salk Institute, P.O. Box 85800, San Diego, CA. and Dept. Anatomy, Univ. of Navarra, Pamplona, Spain.

Previous retrograde tracing experiments demonstrated that the macaque monkey entorhinal cortex receives several direct neocortical inputs (Insausti et al.,1987). In order to analyze the terminal distribution of these projections, discrete injections of ^{3}H amino acids were made into several of the afferent cortical areas. Orbitofrontal and temporal polar cortical regions project relatively widely within several subdivisions of the entorhinal cortex. More restricted projections were observed from the perirhinal cortex (which terminates mainly in the rostral half of the entorhinal cortex), and the superior temporal gyrus, parahippocampal gyrus, and the retrosplenial cortex (all of which preferentially innervate the caudal half of the entorhinal cortex). The lateral field of the entorhinal cortex generally receives a heavier cortical input than other entorhinal fields except when the injection involved the retrosplenial cortex. In this case, heavier labeling was located medial and caudal to the lateral subdivision. In all cases, layer I received the densest terminal innervation, followed by layers III, V and VI. These results indicate that the cortical projections to the entorhinal cortex are diffusely distributed but have a rough topographic organization.

343.7

EVIDENCE FOR A DIRECT CELL-TO-CELL INFLUENCE OF THE HIPPOCAMPUS ON MEDIAL FRONTAL "VISCERAL MOTOR" CORTEX. K.G. Ruit and E.J. Neafsey. Dept. of Anatomy, Loyola Univ. Med. Ctr., Maywood, IL 60153.

Hippocampal (HIPP) stimulation significantly decreases heart rate and blood pressure, and we hypothesize it does so via its connection with the medial frontal cortex (MFC), a region which projects directly to the solitary nucleus (NTS). The present electrophysiological and anatomical study was undertaken to determine the degree to which the HIPP projection to MFC overlaps the origin of the descending projection to NTS.

72 MFC neurons were antidromically activated by NTS stimulation with an average latency of 31 msec (SD = 10). MFC neurons also responded to HIPP stimulation, and, in a few cases, the orthodromic spike from HIPP collided with and eliminated the antidromic spike from the NTS. Following injections of wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) into both the NTS and ipsilateral HIPP, light microscopy showed that the terminal-like labelling from HIPP appeared to overlap the retrogradely labelled cells from NTS in the MFC. Electron microscopy confirmed that HRP-labelled terminals do, in some cases, synapse on HRP-labelled cells. These results indicate that the pathway from HIPP to the MFC cells which project to NTS may be monosynaptic and, therefore, directly influence a central visceral control system. (Supported by Loyola University Potts Estate Fund Grant 842-04).

343.8

CONTRASTING EFFECTS OF STIMULATION OF AMYGDALA AND HIPPOCAMPUS ON SUBPALLIDAL OUTPUT NEURONS TO THE PEDUNCULOPONTINE NUCLEUS (PPN). C. T. Tsai*, M. Wu*, C. R. Yang* and G. J. Mogenson. Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

Hippocampal stimulation inhibited subpallidal(SP) output neurons to the PPN while microinjection of NMDA into the hippocampus increased locomotor activity, suggesting that signals from the hippocampus disinhibit PPN neurons to produce hypermotility (Yang & Mogenson, Neuroscience, 23:1041-1055, 1987). In contrast, microinjection of NMDA into the amygdala suppressed locomotor activity which was also mediated via the SP (Yim & Mogenson, Proc. Can. Fed. Biol. Sci., 30:120, 1987). A comparative study was made to find out whether there is contrasting effect of electrical stimulation of amygdala and hippocampus on SP-PPN neurons identified by antidromic stimulation.

In a total of 50 SP-PPN neurons, only 20 were able to be tested with single pulse stimulation of the amygdala and hippocampus. Fourteen were activated by amygdala stimulation and 6 were inhibited whereas 5 were activated by hippocampal stimulation and 13 were inhibited ($X^2=5.17$, $p<0.05$). These observations provide electrophysiological evidence that the amygdala and hippocampus have opposite effects on SP neurons projecting to the PPN.
(Supported by NSERC of Canada)

343.9

INVOLVEMENT OF N. BASOLATERALIS AMYGDALAE IN ATTENTION IN CAT. M.F.Montaron*, J.J.Bouyer, C.Durand*, P.Delagrange*, A.Rougeul* and P.Buser*. Institut des Neurosciences, Département de Neurophysiologie comparée, CNRS-Université P.& M.Curie, 9, quai Saint Bernard, F-75005 Paris.

In cat the behavior of motionless focalized attention upon a target is accompanied by the development of rhythmic cortical activities at 36 Hz ("beta" rhythms) in the fronto-parietal cortex. Both behavior and accompanying rhythms are controlled by the ventral tegmental area (VTA): bilaterally VTA lesioned cats display hyperactivity in conditions in which normal animals would develop immobility and watching (Montaron et al., *Behav.Brain Res.*,6:129.1982). On the other hand, bilateral lesion of nucleus accumbens (Acc), a relay on the meso-limbic pathway from VTA which also projects to the striatum has the opposite effects, eliciting perseveration of motionless attentive fixation with a high rate of cortical beta rhythms (Bouyer et al., *Exp.Neurol.*,92:698,1986). The VTA is also a source for connections to the amygdala which in turn projects to Acc. With these data in mind, we have now performed bilateral kainic lesions restricted to the basolateral nucleus of the amygdala and controlled the postlesional behavior and beta activity. Both manifestations were markedly decreased after such lesions, but interestingly, no spontaneous motor hyperactivity could be noticed in this case. From this we tend to conclude that in our experimental conditions the amygdala controls the motivational component of the attentive state with no major effect upon its motor behavior component, at variance with the Acc,which being part of the striatal complex and also receiving amygdala influences, may exert a higher level control on attention.

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343.10

ORGANIZATION OF HIPPOCAMPAL EFFERENT PROJECTIONS TO THE CEREBRAL CORTEX IN THE RHESUS MONKEY. G.J. Blatt and D.L. Rosene, Dept. of Anatomy, Boston University School of Medicine, Boston, MA 02118.

To identify the cells of origin of the direct hippocampal formation (HF) projection to the cerebral cortex, injections of retrograde fluorescent tracers were placed in the posterior (PPHG) and anterior (APHG) parahippocampal gyrus and the medial (MFC) and orbital frontal cortex (OFC). Injections in the medial PPHG labeled cells in the subiculum and throughout a central strip of CA1 stratum pyramidale. This strip extended longitudinally the entire anteroposterior length of the HF. Similarly an injection placed laterally in the PPHG labeled a longitudinal strip in the most lateral part of CA1 while injections in the APHG lateral to the rhinal sulcus labeled cells in a more medial strip of CA1. In contrast, MFC and OFC injections labeled cells mainly in the subiculum while in CA1 labeled cells were widely distributed but limited to the deepest part of stratum pyramidale. These results demonstrate that projections from CA1 to the PPHG and APHG originate topographically from longitudinally oriented strips of cells in contrast to projections to the MFC and OFC that originate from the deepest part of stratum pyramidale. These observations suggest a unique functional differentiation in CA1 of the monkey HF.

(Supported by NS19416, NS16841 and AG04321)

343.11

BASAL FOREBRAIN AFFERENTS TO THE HIPPOCAMPAL FORMATION IN THE RHESUS MONKEY. D.L. Rosene, P.L. Heilbroner and M.B. Moss Dept. of Anatomy, Boston University School of Medicine, Boston, MA 02118.

Projections from the magnocellular basal forebrain nuclei to the hippocampal formation (HF) were examined using acetylcholinesterase (AChE) histochemistry and anterograde and retrograde tracers. The medial septal nucleus (MS) and vertical limb of the diagonal band (VDB) projected strongly to uncal levels of the HF, especially to laminae of CA4, CA3, CA2, and the prosubiculum (ProS) as well as the molecular layer of the dentate gyrus (DG). This projection continued throughout the entire length of the HF but was progressively diminished caudally. Like the MS-VDB, the nucleus basalis (NB) projected most heavily to the uncal HF, although less densely. Caudally the only dense NB projection was to the border of the stratum moleculare and stratum radiatum at the junction of CA1 and ProS. These projection patterns correspond closely to the laminar pattern of AChE except in the molecular layer of the DG where the basal forebrain afferents were uniform. Injections of retrograde tracers into the HF confirmed that these projections originated from the MS, VDB and NB. AChE double-labelling demonstrated that from 30 to 50% of the retrogradely labeled neurons were likely to be cholinergic.

(Supported by NS19416, AG04321 and 5T32-NS07152)

343.12

RESPONSES OF MEDIAL SEPTAL/DIAGONAL BAND CELLS TO ELECTRICAL STIMULATION OF THE DORSOMEDIAL-POSTERIOR HYPOTHALAMIC NUCLEI. B.H. Bland*, R.D. Ford*, L.V. Colom, (SPON:A Spira) University of Calgary, Dept. of Psychology, Calgary, Alberta T2N 1N4.

Electrical stimulation of the dorsomedial-posterior hypothalamic nuclei in the urethane anaesthetized rat produces theta (θ) in the hippocampal formation. Furthermore, increases in stimulus intensity are linearly related to increases in θ frequency. This effect is mediated by the medial septum/diagonal band nuclei since after lesions here θ can no longer be produced in the hippocampal formation by such stimulation. The purpose of this study was to investigate the effects of dorsomedial-posterior hypothalamic stimulation on the discharge properties of medial septal/diagonal band cells. Cells were classified along three dimensions, according to previously used criteria (Colom and Bland, Brain Research, 1987), as theta-on or off, phasic or tonic, linear or non-linear, during the spontaneous occurrence of θ and large amplitude irregular activity (LIA). Stimulation did not affect a cell's classification as on or off or phasic or tonic. The major effect was to change phasic non-linear theta-on cells to linear, suggesting that this ascending hypothalamic system plays an important role in increasing the number of septal cells that code the frequency shifts of hippocampal formation theta.

343.13

IDENTIFICATION OF ZINC-CONTAINING NEURONS BY RETROGRADE TRANSPORT OF ZINC-SELENIDE. G. A. Howell and C. J. Frederickson. Lab. for Neurobiology, Univ. of Texas at Dallas, Richardson, Texas 75083

We previously have shown with lesion techniques that the zinc-containing terminals in the BNST, N. terete, and VMH arise from axons of the stria terminalis and fornix/fimbria (Frederickson et al., Soc. Neurosci. Abst., 1986, 12:1532). In the present work, we used a retrograde transport marker for zinc-containing neurons (Danscher, In: Frederickson et al., The Neurobiology of Zinc, Liss, N.Y., p. 185) and sought to identify the specific cells of origin of these zinc-containing pathways.

Selenite ions (Na_2SeO_3) were infused into BNST/VMH regions in anesthetized rats, causing ZnSe to precipitate in situ in zinc-containing terminals . Twenty-four hours later, rats were sacrificed, and cryostat sections were developed in neo-Timm's, rendering the ZnSe visible by silver encapsulation.

Staining at the injection site showed characteristic labeling of the neuropil (presumably the zinc-containing boutons) with no labeling of perikarya. However, dense and selective labeling of individual perikarya was found in the ipsilateral amygdalohippocampal area, certain amygdalar nuclei, and in the ventral subiculum. These findings suggest that retrograde transport of precipitated zinc (ZnSe) is a chemospecific marker for identifying and mapping zinc-containing neuronal systems. Supported by MH 42798.

343.14

THE FUNCTION OF A ZINC METALLOTHIONEIN FROM BOVINE HIPPOCAMPUS, **V. K. Paliwal* and M. Ebadi. Dept. of Pharmacol., Univ. of Neb. Coll. of Med., 42nd St. and Dewey Ave., Omaha, NE 68105**

Mammalian hippocampi not only contain high concentrations of dithizone chelatable zinc, but also exhibit regional variation in this essential element, with concentrations being highest in the hilar region and lowest in the fimbria. For example, the concentration of zinc in the mossy fiber axons has been estimated to approach 300-350 μM. In an attempt to investigate further the dynamic metabolism of zinc, we have searched for and have identified a metallothionein-like protein in bovine hippocampus with the following properties: similar to the zinc-induced hepatic metallothionein (hep-MT), the hippocampal metallothionein-like protein exhibits an elution volume (Ve/Vo) of 2.0 on gel filtration chromatography, and produces two isoforms, which on a reverse phase high performance liquid chromatography (HPLC) show retention times of 16.72 min. (hep-MTI produces 16.53 min.) and of 17.94 min. (hep-MTII produces 18.45 min.), respectively. The hippocampal metallothionein isoform II contains a cysteine to zinc ratio of 2.8 to 1.0, and, as shown by studies involving UV spectral analysis, apparently lacks aromatic amino acids, but possesses metallomercaptide bonds. The results of these studies suggest that the metallothionein may play an essential role in regulating the transport and/or accumulation of zinc in the hippocampus. Investigations to determine the regional CNS localization, as well as the subhippocampal distribution of this metallothionein-like protein, are in progress.

(Supported in part by a grant from USPHS ES-03949.)

343.15

COLOCALIZATION OF GABA AND PEPTIDES IN THE RAT BASOLATERAL AMYGDALA. A. J. McDonald and J. C. Pearson. Departments of Anatomy, Univ. of South Carolina Sch. of Med., Columbia, SC 29208, and Wright State University Sch. of Med., Dayton, OH 45435.

Although previous studies have shown that GABA and peptides are found in nonpyramidal neurons of the basolateral amygdala, it is not known whether these substances co-exist in the same neurons. The present study utilized a two color ABC immunoperoxidase procedure (with DAB and BDHC as chromogens) to investigate this question. The anti-GABA antiserum was raised in a guinea pig while the antibodies to peptides were raised in other species. Most somatostatin-positive and neuropeptide Y-positive neurons were also GABA-positive. All large CCK-positive neurons appeared to contain GABA while a subpopulation of small CCK cells, found mostly in the lateral nucleus, were mostly GABA-negative. Some VIP containing neurons also contained GABA. Thus, it appears that many neurons in the basolateral amygdala that contain the inhibitory transmitter GABA also contain putative peptide neuromodulators. On the other hand, most GABA-positive cells were not double-labeled. These findings are very similar to those obtained in studies of the cerebral cortex and provide further support for the notion that the cortex and basolateral amygdala share many important features. (Supported by NIH Grant NS 19733).

343.16

CHEMICAL ANATOMY OF THE AMYGDALA IN PRIMATE. A.F. Sadikot*, Y. Smith and A. Parent. Lab. of Neurobiology, Fac. of Med., Laval Univ., Québec, Canada.

Very little information is yet available on the localization of neurotransmitters in the amygdala of primates. Thus, the peroxidase-antiperoxidase immunohistochemical method was used to stain serial coronal sections of the temporal lobe of 2 squirrel monkeys (*Saimiri sciureus*) with polyclonal antibodies to tyrosine hydroxylase (TH), serotonin (5-HT) and met-enkephalin (ENK), and with a monoclonal antibody to substance P (SP). SP-immunoreactive cell bodies were noted in the basolateral nucleus and, more rarely, in the central nucleus of the amygdala. SP terminals and fibers of fine caliber occurred in the dorsal region of the medial nucleus as well as in the central and basolateral nuclei. Numerous ENK-immunoreactive fibers of the woolly type were seen in the lateral portion of the central nucleus and, to a lesser extent, in its medial portion which also contained many thinner axons and scattered terminals. 5-HT immunostaining in the amygdala was widespread with varicosed fibers being particularly prominent in the basal, lateral and central nuclei. 5-HT terminals were visualized in the central nucleus of the amygdala and the periamygdaloid cortex. TH immunoreactivity was seen mainly as scattered terminals in the central nucleus and fine varicosed fibers in the lateral and basal nuclei. The present study provides the first description of the distribution of SP, ENK, 5-HT, and TH in the amygdala of primates. These results should serve as a more rational basis for defining amygdaloid nuclei in mammals and as a framework for future studies of the chemospecific connections of the amygdala in primates. (Supported by grants of the MRC, FRSQ and FCAR).

LEARNING AND MEMORY: PHYSIOLOGY IV

344.1

MEMORY FIELDS: DIRECTIONAL TUNING OF DELAY ACTIVITY IN THE DORSOLATERAL PREFRONTAL CORTEX OF RHESUS MONKEY. S.Funahashi, C.J.Bruce, and P.S.Goldman-Rakic Sec. Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

The prefrontal cortex (PFC) in nonhuman primates is essential for the spatial working memory processes tapped by delayed response tasks. Our previous study using an oculomotor delayed response paradigm provided evidence that PFC neurons are active (excitation or inhibition) in the delay period only when visual cues were presented at specific locations in the visual field (Funahashi et. al., Soc. Neurosci. Abstr., 12:554, 1986). We termed the area of the visual field where a neuron shows prominent delay activity as that neuron's memory field.

In the present experiment, we quantitatively analyzed the spatial tuning of these memory fields. Eight visual cues separated by 45 deg in polar direction at an eccentricity of 13 deg were presented randomly in the cue period of the oculomotor delayed response task. Tuning curves were made based on the average discharge rate during the delay period for each cue. The best directions were estimated from the parameters used to fit these experimental data to a Gaussian function. Tuning indices were defined as the departure from the best direction which reduced the response by 50% along the fitted curve.

Seventy-six PFC neurons with directional delay activity were analyzed. Although the best directions were widely distributed, a strong contralateral bias was evident: 62% of the best directions were toward the visual field contralateral to the neuron's hemisphere whereas only 26% were toward the ipsilateral field. The remaining 12% of the neuron's best directions were around the vertical meridian. The tuning indices were distributed widely (3 to 133 deg) with median of 30.4 deg; however, about half the neurons had indices between 20 and 40 deg and 9% of neurons showed tuning narrower than 10 deg.

Our results indicate that PFC neurons have a range of memory fields, with each hemisphere primarily mapping the contralateral hemifield. The findings that most memory fields are comparable to visual receptive fields and movement fields in the posterior parietal cortex or the superior colliculus suggest that the PFC is closely linked to the brain's centers for spatial vision.

344.2

CHANGES IN HAND KINEMATICS DURING A QUANTIFIABLE LEARNING PARADIGM IN PRIMATES. C.L. Ojakangas, D.K. Onstott, D.C. Tam and T.J. Ebner. Depts. Neurosurgery and Physiology, Neuroscience Grad. Prog., Univ. of MN, Mpls., MN 55455.

A paradigm involving a 2-dimensional, visually guided arm movement was developed. Monkeys moved a cursor via a manipulandum on a video screen from a start box to one of 4 randomly presented target boxes. The relationship between hand and cursor was the same for the control phase (1.0) and was varied during learning. Movement strategies during adaptation to 4 gains (.6 - 2.0) were studied via control, learning and testing phases. Results indicated that in learning a new gain monkeys scaled the velocity of their movement to match the distance the hand needed to travel (i.e. further distance, higher velocity) ($p<.01$, 2-tailed T test). Time to maximum velocity remained relatively constant over phases and across gains ($p>.01$), as did reaction time. With time constant, distance until 1st (maximum) velocity peak varied directly with gain change. During learning, the 2nd velocity peak seen initially was eliminated and hand trajectory smoothed. Total movement duration decreased ($p<.01$) although remained greater than control for smaller gains once learned ($p<.01$). Results support the hypothesis that movement duration is a free variable and invariant during the first phase of movement. The learning strategy consists of scaling hand velocity to match gain. Supported by NSF/BNS-8707572 and NIH/NS-18338.

344.3

ACTIVITY OF MONKEY HIPPOCAMPAL AND ENTORHINAL NEURONS DURING LEARNING. T. Ono, R. Tamura*, E. Tabuchi* and M. Fukuda*. Dept. Physiol., Fac. Med., Toyama Med. & Pharmaceu. Univ., Sugitani, Toyama 930-01, JAPAN

Lesion in the monkey hippocampus produces spatial memory deficit. Neuronal activity in the monkey hippocampus and entorhinal cortex was recorded during operant feeding, drinking and shock avoidance, delayed matching to sample with response-delay, and a clinical test with visual, auditory presented from various directions. Focus in the clinical test was on neuronal response selectivity to direction. Of 1075 neurons tested, 581 (54.0%) responded to some task or the clinical test and 399 (37.1%) were identified as hippocampal or entorhinal. Of these, 40 (3.7%) were direction specific and many of these were in the caudal hippocampal formation. Of these 40 neurons, 27 responded only to visual stimuli (left anterior, 4; right anterior, 22; multidirectional, 1), 8 responded only to auditory stimuli (anterior, 1; right posterior, 2; posterior, 3) and 5 responded to both visual and auditory stimulation (right anterior, 1; multidirectional, 4). The direction-selective neurons tended to respond to visual stimuli presented from the right anterior, and to auditory stimuli presented from the posterior direction. The results suggest hippocampus and entorhinal cortex involvement in central processing of directional or spatial information.

344.4

CATECHOLAMINE SENSITIVITIES OF PREFRONTAL NEURONS RELATED TO A DELAYED RESPONSE TASK OF RHESUS MONKEYS. T. Sawaguchi, M. Matsumura* and K. Kubota. Dept. of Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

Influences of iontophoretically applied dopamine (DA) and noradrenaline (NA) on neuronal activities related to a delayed response task were examined in the prefrontal cortex. The task was started by monkeys rotating a handle to a central zone, and consisted of pre-cue (central lamp, 1 s), cue (left or right lamp, 1 s), delay (4 s), go (red lamp, rotating the handle to left or right zone within 1 s), and reward periods. Prefrontal neurons showed changes in activity during the pre-cue period (PC-types, n=18), both the cue and go periods (Cue/GO-types, n=16), the go period (GO-types, n=16), and the delay period (Delay-types, n=64). Delay-types consisted of Differential neurons (n=33) whose activity differed between left- and right-cue trials, and Non-Differential neurons (n=31). DA increased the activity of most of Cue/GO- (16/16), GO- (13/16) and Delay-types (49/64), and NA inhibited the activity of most of PC-types (13/18) and Non-Differential Delay-types (25/31). Fluphenazine and haloperidol attenuated changes in activity during the cue, delay and go periods, while sulpiride had no clear effects. Results suggest that DA auguments prefrontal neuronal activity involved in temporal integration of visual cue and motor performance, and NA inhibits prefrontal neuronal activity related to visual reception.

344.5

SELECTIVITY OF DELAY DISCHARGES TO PICTORIAL PATTERNS IN A DELAYED MATCHING-TO-SAMPLE TASK IS MAXIMIZED THROUGH LEARNING IN NEURONS OF THE PRIMATE TEMPORAL CORTEX. Y.MIYASHITA Dept.of Physiol., Univ.of Tokyo, Tokyo, Japan.

Memory of visual experience in the primate has been proposed to be stored in the neural network of the temporal cortex. Recently, shape-selective working memory neurons were found in the monkey temporal cortex (1). I report here evidence suggesting that the responsiveness of these neurons is formed through learning. Two monkeys (Macaca fuscata) were trained for 2 months to perform a modified delayed matching-to-sample task; the sample and match stimuli were presented successively on a video monitor, each for 0.2s at a 16s delay interval. A set of 100 computer-generated colored fractal patterns was used for training each monkey (learnt stimuli). When extracellular discharge of a neuron was recorded in the anterior ventral temporal cortex of these monkeys, not only the 100 learnt patterns but also another set of 100 patterns, newly generated for each neuron (new stimuli), were presented. Eighteen neurons with shape-selective sustained delay discharge were thus tested. In all of them, the maximum delay discharge among those induced by the 100 learnt stimuli was stronger than that induced by the 100 new stimuli. The stimulus-selectivity was also higher in the learnt stimuli than in the new stimuli in 17 of the 18 cells. The observations suggest that the neurons are involved in a long-term pictorial memory process.

(1) Miyashita,Y. & Chang,H.S. Nature 331,58-60,1988.

344.6

EFFECTS OF GLUCOSE ON HUMAN MEMORY. N. P. Azari* (Spon: J. F. Masken). Psychology Dept., Colorado State University, Ft. Collins, Colorado 80523.

Animal studies have shown memory enhancement following post-trial administration of intermediate doses of glucose as well as amnesic effects resulting from glucose doses less than or greater than the optimal dose. One study has investigated memory improvement effects of glucose in elderly humans (Gonder-Fredrick, L., Hall, J. L., Vogt, J., Cox, D. J., Green, J., & Gold, P. E., Physiol. & Beh., 41, 1987). This experiment tested the hypothesis that moderate doses of glucose may improve memory in healthy humans. 18 males (18-25 yrs.) received 3 doses (0, 30, 100 g) of glucose orally (300 ml beverage) in a random, double-blind, aspartame-controlled, triple crossover design. 30 min. post-beverage subjects saw 40 nouns on a computer and took a recall and recognition test. Blood samples were drawn every 15 min. over the hour for blood glucose (BG) analysis. Data were divided according to time of peak BG change (BGC). Subjects given 30 g glucose with peak BGC at test time showed recognition memory improvement from the 0 g to 100 g treatment ($p \leq 0.05$); subjects without peak BGC at test time showed no differences. Subjects with peak BGC after test time given 30 or 100 g glucose showed negative correlations between BGC and recognition scores (r's ≥ 0.6, p's ≤ 0.05). These results confirm the importance of glucose in memory modulation.

344.7

MEMORY-SPECIFIC CHANGES IN INTERSPIKE INTERVALS IN DOMINANT HUMAN TEMPORAL LOBE. D. F. Cawthon,* E. Lettich,* O. D. Creutzfeldt,* G. A. Ojemann. Dept. Neurosurgery, Univ. of Washington, Seattle, WA 98195; Dept. Neurobiol., Max Planck Inst. of Biophys. Chem., Göttingen, F.R.G.

During epilepsy surgery, patients undergoing microelectrode extracellular recordings from dominant temporal lobe, under institutional rules for human subjects, have engaged in simple naming, reading and spatial matching tasks and a post-distractional short-term memory task. The most common neuronal firing pattern during the memory task was an increase in frequency during both silent input and overt retrieval phases (Ojemann et al. Brain, 1988, in press; Cawthon et al., in preparation), outlasting overt and presumably silent speech by several seconds. We have now examined changes in interspike intervals (ISIs) during the memory task in patients with well-isolated single cell records. In two patients, increases in the shortest band of ISIs present (5-30 msec in one, 40-120 msec in another) accompanied the increased frequency but also showed some dissociation from frequency and greater specificity to the memory task. Short ISIs increased discretely during some subdivisions of memory phases when frequency remained tonically elevated but unchanging, or when frequency actually dropped. These changes specific to short ISIs may be the mechanism for informational coding of linguistic and memory features within a task. (Supported by NIH Grants NS21724, NS20482, NS17111 and NS07289).

344.8

MODULATION OF HUMAN EVENT-RELATED POTENTIALS BY WORD REPETITION - EFFECTS OF TEMPORAL LOBE PATHOLOGY. M.D. Rugg*, M.E. Nagy*, and R.C. Roberts* (SPON: European Brain and Behaviour Society) Dept. of Psychology, University of St Andrews, U.K. and Dept. of Medicine, University of Dundee, U.K.

It has been reported that the modulation of event-related potentials (ERPs) by word repetition is attenuated after left temporal lobectomy (Halgren and Smith, Hum. Neurobiol., 6: 129-139). We provide further data concerning the relation between temporal lobe pathology and the 'ERP repetition effect'. ERPs were recorded from multiple scalp electrodes while subjects monitored a series of visually presented words for the occasional occurence of non-words. In patients with primary generalised or right-sided temporal lobe epilepsy, repeated words evoked ERPs which were typically some 5 μV more positive-going 300-600 msec post-stimulus than ERPs to non-repetitions. While some patients with left-sided temporal lobe epilepsy showed an abnormally small repetition effect, in the majority it was normal. Patients with left temporal lobectomies showed a variable pattern, ranging from abnormally small effects at all scalp sites to effects of the same size and scalp distribution as in controls. These data indicate that although abnormalities in the 'ERP word repetition effect' are associated with damage to the left temporal lobe, they are not a necessary consequence of such damage.

344.9

PREFRONTAL UNIT ACTIVITY DURING THE ASSOCIATIVE LEARNING. M. Watanabe. Dept. of Liberal Arts, Tokyo Engineering Univ. Katakura-cho, 1404 Hachioji, Tokyo, 192 JAPAN

Two monkeys were trained on a reaction time task overlapped with a classical conditioning paradigm. Sequential events of the task were as follows; (1)animal's pressing response of the lever; (2)presentation of a visual or auditory cue; (3)delay period of 1 sec.; (4)imperative stimulus presentation; (5)release of the lever by the animal. The visual or auditory cue signaled whether or not a drop of fruit juice would be given for the animal's releasing response. In this task, the animal had to release the lever even on the trial where no juice was given, to advance to the next trial.

Single unit activity was recorded from the prefrontal cortex while the animal was performing the task, in order to clarify whether prefrontal units are involved in coding the meaning or associative significance of the stimulus in relation to whether a certain cue is associated with the juice reward. Of 436 task-related units obtained, 68 units showed differential activity in relation to the associative significance of the visual or auditory cue, and 50 units showed such differential activity for both modalities of cues. The results indicate that prefrontal units are involved in coding the associative significance of the stimulus, and that in the majority of these units such coding is done in modality specific and in some units across modalities.

344.10

SHORT AND LONG LATENCY BLINK CRs ARE SUPPORTED BY ACTIVITY OF UNITS IN THE MOTOR CORTEX AND THALAMUS OF CATS. C.D. Woody, S. Aou, E. Gruen*, D. Birt, O. Melamed*, and J. Wangwongvivat*, UCLA Med. Ctr., Los Angeles, CA 90024.

Recent ablation studies suggest that long latency eyeblink conditioning depends on cerebellum and subcerebellar nuclei for its development. We examined the hypothesis that higher brain structures might support such conditioning in normal, intact animals. Recordings from the motor cortex of awake cats disclosed patterns of unit activity that increased to support performance of Pavlovian blink conditioning with four separable latency components of 8 - >112 ms onset following delivery of click CS. The components developed pari passu with those of the conditioned response, the latter measured electromyographically, and were associated with increases in unit excitability to intracellularly injected depolarizing current. Recordings from intralaminar and lateralis dorsalis thalamic nuclei also found increases in activity of short and long latency correlated with development of conditioning. Further studies demonstrated conditioning of short and long latency activation of single units of the motor cortex after pairing click CS with tap US and local iontophoretic application of glutamate. Associated with development of unit CRs were increases in neuronal excitability and input resistance and a decrease in a current resembling K_A measured by single electrode voltage clamp techniques. Ablation of the motor cortex of cats prevents short but not long latency blink conditioning. (Supported by NS25510/HD05958.)

344.11

LEARNING-RELATED CHANGES IN SINGLE-UNIT ACTIVITY IN THE MEDIAL PREFRONTAL CORTEX (PFCm) OF RABBITS. C. M. Gibbs. Neuroscience Lab., WJB Dorn Veterans' Hosp., & Dept. of Psychology, Univ. of South Carolina, Columbia, SC 29201.

Bilateral lesions of the PFCm severly disrupt the development of discriminative bradycardiac CRs in rabbits (Buchanan SL and DA Powell: JCPP 96:755), and tone (CS)-evoked multiple-unit activity in the PFCm undergoes training-induced changes that are reliably correlated with aversively conditioned changes in heart rate (Gibbs CM and DA Powell: Brain Res 442:86). Accordingly, we have begun evaluating single-unit activity in the PFCm during or following either simple Pavlovian conditioning (tone CS paired with eyeshock US), differential conditioning (one of two tones paired with eyeshock) or nonassociative training (e.g. unpaired stimuli).

Our results to date have indicated the PFCm consists of numerous, widely interspersed cell populations differing from one another with respect to the patterns of their maintained, CS- and US-evoked activity. Our data have also suggested that at least three of these functional populations show associative training-induced changes in their CS-evoked discharge that are consistent with the concomitant development/expression of bradycardiac CRs. These data thus provide further support for the suggestion that the PFCm participates in learned cardiovascular adjustments in rabbits. (Supported by VA Institutional Research funds)

344.12

INFLUENCE OF MICROIONTOPHORETIC CHOLINERGIC DRUGS ON CONDITIONED CORTICAL UNITS. K. Turco*, D. Yadon* and J. Pirch. Dept. of Pharmacology, Kirksville Coll. Osteo. Med., Kirksville, MO 63501

Microiontophoretic techniques were used to study the effects of cholinergic agents on rat frontal cortex neurons which demonstrated a differential response to reinforced (CS+) and non-reinforced (CS-) tone stimuli. Medial forebrain bundle (MFB) stimulation served as reinforcement. Cortical slow potentials were used to monitor conditioning. A current range was determined from self-stimulation testing for use in tone discrimination training. Tone duration was 2 sec, and MFB stimulation was applied at the termination of CS+. Trained animals were anesthetized with urethane, and units with a differential response to CS+ were characterized in detail. Of the 34 units in this study, 23 exhibited an excitatory response to CS+; 21 of these were excited by ACh. Atropine or tropicamide antagonized both the CS+ and ACh excitatory responses in 15 of these units, whereas in 6 units only the ACh response was antagonized. Two units that showed an excitatory response to CS+ demonstrated no response to ACh, but the CS+ was antagonized by atropine. CS+ inhibited 11 units; 10 of these were excited by ACh. Atropine or tropicamide antagonized both the CS+ and ACh responses in 6 of these units, in 3 units only the ACh response was antagonized, while in 1 unit there was no antagonism of either the ACh or CS+ response. One unit inhibited by CS+ was also inhibited by ACh; no antagonism of the CS+ was found in this unit, and antagonism of ACh was equivocal. ACh may be involved in the conditioning-related neural responses of a significant population of cortical units. Other neurotransmitters, however, may be necessary to explain the responses of units described here that demonstrate inhibitory responses and/or resistance to the effects of the cholinergic antagonists. Supported by NIH grant NS22408.

344.13

LEARNING SPECIFICALLY ALTERS FREQUENCY RECEPTIVE FIELDS IN AUDITORY CORTEX OF GUINEA PIG. J. Bakin,* C.D. Condon* and N.M. Weinberger. Cntr. Neurobio. Learning & Memory and Dept. Psychobiology, Univ. California, Irvine CA 92717.

Acquisition of the classically-conditioned pupillary dilation response in muscle-blocked cat is accompanied by specific changes in the frequency receptive fields (FRF) of single neurons in auditory cortex at the frequency of the conditioned stimulus (Diamond & Weinberger, Brain Res., 1986, 372, 356-60). The present study investigated the effects of both classical and instrumental avoidance conditioning on the FRF of clusters and single neurons in the auditory cortex of undrugged guinea pig.

FRF were determined before and following classical (body movement) or avoidance conditioning (wheel running) of male adult guinea pigs bearing chronically implanted microwires in auditory cortex. Conditioned stimuli were single tones at non-best frequency. FRF were determined in a chamber separate from the training environment. Both classical and instrumental procedures resulted in alterations of FRF for both clusters and single neurons; in most cases, changes were maximal at or immediately adjacent to the CS frequency. The findings indicate that learning-induced frequency-specific changes in tuning extend to various orders of animals, preparations and training.

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344.14

THALAMOCORTICAL, HIPPOCAMPAL AND AUDITORY NEURONAL ACTIVITIES RELATED TO AUDITORY WORKING MEMORY PROCESS IN THE RAT. Y. Sakurai. Dept. of Psychology, Toyama Med. & Pharmaceu. University, Sugitani, Toyama 930-01, JAPAN.

The present study was concerned with thalamocortical, hippocampal and auditory neuronal systems underlying auditory working memory in the rat. The task was an auditory continuous nonmatching-to-sample (Sakurai, Psychobiol., 15:277, 1987). Pressing a panel (Go) was rewarded if the tone for the current trial was different than the tone for the preceding trial. A 3s of delay period was imposed between trials. In the tone-presentation period on preceding trials, the entorhinal cortical (EC) and the dorsomedial thalamic (DMT) units showed differential activation regarding type of the tone (high or low) and outcome of the next response (correct or error) respectively. In the delay period, the DMT units showed differential activity related to outcome of the next response. In the tone-presentation periods immediately prior to response, the medial geniculate body (MGB) units showed tone-differentiated activity. The MGB, CA1, CA3, EC, dentate gyrus and motor cortex had more units showing differential activation related to type of the next response (Go or No-Go). A macro neuronal model underlying the auditory working memory will be discussed. (Supported by Grant-in-Aid for Scientific Research 62710039 from the Japanese Ministry).

344.15

SHORT TERM MEMORY PROCESS IN THE PARIETAL EYE. D.J. Eder and R. K. Rader. Biological Sciences, Southern Illinois University, Edwardsville, IL 62025 and Searle Pharmaceutical, Chesterfield, MO 63198.

The lizard parietal (pineal) eye contains conelike photoreceptors that synapse directly onto ganglion cells. We have recorded characteristic electroretinographic waveforms evoked by long- and short-wavelength light stimuli in anesthetized lizards, Anolis carolinensis. Response amplitudes and waveforms evoked by identical 2-sec. blue test flashes varied depending on whether they followed either a red (589nm) or blue (460nm) 5-sec. conditioning stimulus. The conditioning effect persisted for more than 20 min. and was elicited by conditioning intensities too low to bleach quantities of photopigment sufficient to reduce quantum catch. After a red conditioning stimulus, dark recovery of the blue system progressed with a time constant of 8 min. at 24° C. The blue test stimulus itself immediately completed the recovery process by restoring full sensitivity to subsequent blue flashes. Subsequent and test flashes suppressed blue responses and "reset" the system. This eye thus seems to "remember" the wavelength of the last flash presented to it. The memory process depends on a photic "switch" and follows a time course resembling that of short term memory. We interpret the results by postulating synaptic interaction of at least two neurotransmitter systems at the ganglion cell level.

344.16

LEARNING MECHANISM FOR IDENTIFICATION OF ACOUSTIC FEATURES. B.S. Seebach* and N. Intrator*. (SPON: L. N Cooper) Center for Neural Science and Division of Applied Mathematics, Brown University, Providence, RI 02912.

A biologically-plausible neural network model is proposed which learns to identify acoustical signals by recognizing the presence or absence of various signal components, rather than by recognizing the signals as whole units. The presence of rich component structures in naturally-occurring signal sets suggests that this type of learning is common for perceptual systems.

Acoustical inputs to the network undergo pre-processing designed to mimic cochlear processing to a first approximation, using bandpass filters and zero-crossing analyzers to produce an energy vs. frequency representation of the signal for each time period. These representations are combined with the analog waveform, then input to first order neurons through a series of delay mechanisms, in a manner similar to Licklider (1951), producing a graded tonotemporal mapping of acoustical events in these neurons.

The neural network is built of Hebbian-type neurons with the modification introduced by Bienenstock, Cooper, and Munro (1982), which causes each neuron to become selective for one type of input pattern. These neurons are arranged into groups. Competition is induced by a form of global inhibition.

When the number of signals input to the system exceeds the dimensionality of the network, inter-group competition in the network causes groups to begin responding to components (or features) of the inputs in the signal set, rather than to inputs as whole units. Resulting is a demonstration of a biologically-plausible theory of learning and memory giving rise to a psychologically real phenomenon of signal component detection.

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344.17

COMPUTER SIMULATIONS IN NEUROSCIENCE: PSEUDO PSEUDO-RANDOM NUMBERS. C.F. Mactutus and R.M. Booze, Dept. Physiol. Pharmacol., Bowman Gray School of Medicine, Winston-Salem, NC 27103

A reliable source of uniform random numbers is an essential building block for any computer simulation program involving a stochastic process. Many system supplied pseudo-random number generators are *linear* (or mixed) *congruential generators* (*LCG*), which generate a sequence of integers via the relation $I_{n+1} = A * I_n + C$ mod M where M is called the modulus and A and C are positive integers called the multiplier and increments, respectively. A *multiplicative congruential generator* (*MCG*) refers to the same equation, but with $C = 0$. We evaluated a *LCG* found on many personal computers running BASIC, and a *MCG*, recoded in BASIC, but more commonly found on mainframe computers. The present observations summarize three aspects of these generators on an 8 MHz computer: speed, period, and uniformity. Speed of the *LCG* was faster (0.9 msec/sample) than that of the *MCG* (1.06 msec/sample). The period of the *LCG* was 2^{24} (16,777,216 samples; 4.2 hr) relative to $2^{31} - 2$ (theoretically 2,147,483,646 samples; > 26 days) for the *MCG*. Uniformity was assessed by calculating 1000 $X^2_{(99)}$ values, each based on a 1000 number sequence generated from a different seed. A $X^2_{(99)}$ test, performed on these 1000 X^2 values indicated that reasonable proportions of large and small X^2 values were noted, as expected under "true randomness", for the *MCG* ($p < .50$), but not for the *LCG* ($p < .01$). These data 1) suggest that improvements in pseudo-random number generation quality may be obtained with modest decrements in speed, and 2) urge caution in the use of any pseudo-random number generator simply because it is readily available.

344.18

DEVELOPMENT AND CALIBRATION OF A CRYOPROBE FOR USE IN FREELY BEHAVING RATS. S. Campeau* and M. Davis. Dept. of Psychiatry, Yale University, School of Medicine, 34 Park St., New Haven, Ct 06508.

The ability to produce reversible inactivation of localized brain regions would provide an important tool for analyzing neurobehavioral function. Capitalizing on the advantages of cryogenic neural inactivation (Brooks, Rev. Physiol. Biochem. Pharmacol., 95:1-109, 1983), a cryoprobe was developed and calibrated for chronic implantation in freely behaving rats. The probe is made of 24 g stainless steel tubing enclosed within 19 g tubing. A 37 g insulated wire serves as a heating coil and is wrapped around the outer tubing except for its tip. Temperature at the tip of the probe is monitored by an internal thermocouple and controlled by circulating cold methanol at various flow rates. Initially, tissue heat loss was evaluated by acutely implanting the probe in the cerebellum of anesthetized rats. Tissue temperature, measured at various distances around the probe, was found to rise exponentially with distance. To test the effectiveness of the probe physiologically, hindlimb flexion produced by electrical stimulation of the motor cortex was examined in ketamine anesthetized rats. Reversible blockade (tested for up to 30 min and occuring within 30 sec) was observed at tissue temperatures of 5 to10 C when the probe was situated less than 0.7 mm from the pyramidal tract. Moderate hindlimb flexion disruption was obtained when the probe was further away from the pyramidal tract or with milder temperatures. To study blockade of synaptic transmission, rats were chronically implanted with the probe aimed at the ventral nucleus of the lateral lemniscus (VLL), part of the primary acoustic startle circuit. Cooling the VLL reversibly blocked the startle reflex without disrupting ongoing behavior. Startle recovered within 30 sec when cooling was turned off. These results demonstrate the specificity, reversibility, and rapidity of neural inactivation produced by a cryoprobe that can be implanted in freely behaving rats.

344.19

DISCRIMINATIVE CONDITIONING OF AUTONOMIC AND SKELETAL RESPONSES UNDER CONTINUOUS (30-90 DAY) NEUROMUSCULAR BLOCK. B.R. Dworkin and S. Dworkin*. Dept. Behavioral Science. Penn State University College of Medicine. Hershey, PA 17033.

Rats with bilateral silastic tibial n.electrode cuffs are chronically paralyzed and ventilated using a continuous infusion of alpha-bungarotoxin. They have normal blood gases, pH, Na, K, serum protein, hematocrit, blood pressure, heart rate, vasomotor tone and tibial n. activity. The physiological variables, EEG spectra and cortical EP's reflect regular sleep wakefulness cycles and responsiveness to mild stimuli. Following stabilization (3-5 days), equal-intensity auditory stimuli (2kHz; 4 kHz tones) are presented. Habituation is demonstrated within 24 hours and then one tone becomes the CS- and the other a CS+, which terminates in a brief tail shock. All rats showed differential responses of the tibial nerves, and vasoconstriction, blood pressure elevation, bradycardia and EEG desynchronization. The CS+/CS- difference was large and highly reliable ($p<.001$) for all measures. The pattern of responses to the the CS+ are similar to the responses to an aversive stimulus in a non-paralyzed animal. The preparation provides an unusual opportunity to study the physiology of conditioning in several different response systems in a stable chronic preparation.

344.20

RETENTION OF A BACKWARD CLASSICALLY CONDITIONED REFLEX RESPONSE IN SPINAL CAT. J. E. Hoover* and R. G. Durkovic (SPON: F. T. Llados). Dept. of Physiol., SUNY Health Sci. Ctr., Syracuse, NY 13210.

The purpose of this study was to conduct a specific test for retention of a backward conditioning (BC) response in the ischemically decapitate spinal cat preparation. Isometric muscle tension recordings (tibialis anticus) were used to quantify alterations in the magnitude of the flexion reflex during and after backward pairing of superficial peroneal n. stimulation (30 Hz, 0.5 sec), the US, with saphenous n. stimulation (10 Hz, 1.5 sec), the CS. Both US and CS were supramaximal for activation of A-delta fibers. Experimental animals received 30 paired trials (US preceeded CS by 0.25 sec) with an ITI of 3 min. Control animals received the same stimuli but in an explicitly unpaired manner. Following acquisition, all animals received 30 additional CS-alone trials at 5 min. intervals. Conditioned responding was monitored throughout acquisition with CS-alone "probe" trials which tested the excitability of spinal reflex circuits activated by A-alpha or A-alpha plus A-delta fibers.

In confirmation of previous results [J. Neurosci. 6: 2921], BC treatment in this preparation resulted in flexion reflex facilitation. Independent t-tests indicated that significant differences existed between the overall acquisition means of paired and unpaired groups [$t(12) = 3.337$; $p < 0.005$]. Analysis of the A-alpha CS probes yielded similar results. At intensities supramaximal for A-delta fibers, CS-alone trials, following acquisition, resulted in rapid CR decrement toward control group levels. In contrast, analysis of the overall extinction means obtained with A-alpha CS-alone probes indicated evidence of retention of the CR [$t(12) = 2.800$; $p < 0.01$]. These latter findings represent the first demonstration of retention of BC effects in this preparation. Thus, in the spinal cat, both forward conditioning (FC) [Behav. & Neural Bio. 43:12] and BC procedures result in long-term reflex potentiation. *However, unlike FC, which requires activation of A-delta pathways for retention of the CR, this study demonstrates that BC reflex alterations are limited to the spinal circuitry activated by A-alpha fibers.* These and other recent findings [J. Neurosci. 8:502] support the hypothesis that FC and BC are unique phenomena under the control of different neural processes. Supported by NSF grants BNS 8415917 and BNS 8808495.

EPILEPSY IV

345.1

MK-801 PREVENTS SEIZURE RELATED BRAIN DAMAGE FROM PILOCARPINE AND LITHIUM-PILOCARPINE EPIDURAL WELL INDUCED SEIZURES. D.B.Clifford, A.Benz*, J.W.Olney, C.F.Zorumski. Depts.of Neurology and Psychiatry, Washington Univ. Sch. of Med., St. Louis, MO 63110. (SPON:R.Sundermann)

Cholinergic seizures induced by systemic pilocarpine(PILO) have been used as a model of limbic seizures. They are augmented by lithium(LI) pretreatment. We have demonstrated that PILO in high doses will cause seizures and brain damage in an epidural well model. We here studied the modification of focal seizures by LI pretreatment, and the ability of the atropine or MK-801 to protect the animals from seizures or brain damage.

EEG and behavioral observations were made on rats while PILO was instilled in epidural wells. Without pretreatment (N=8) or with LI 3 mEq/kg pretreatment (N=12), the mean EEG seizure threshold dose was 47.5 or 43.4 mM PILO respectively, while the behavioral seizure threshold dose was 101.25 mM or 50 mM PILO.

MK-801, a non-competitive N-methyl-D-Aspartate antagonist was administered sc(N=7, 1mg/kg sc) one hour prior to PILO 200 mM (N=8), or LI/PILO (N=7)in a dose sufficient to cause behavioral seizures. MK-801 protected the brains from the seizure induced brain damage syndrome seen in control rats, but not electrical or behavioral seizures. Atropine 10 mM in the well or 50 mg/kg sc similarly protected the animals. We conclude that LI augments PILO action in the focal epidural well model. Atropine or MK-801 block seizure related brain damage without necessarily blocking electrical seizures. *Supported by DAMD 17-86-C-6010 (JWO). MK-801 generously supplied by Merck Sharp and Dohme.*

345.2

NMDA ANTAGONISTS AND EPILEPTIFORM BURSTS EVOKED BY BICUCULLINE METHIODIDE (BMI) IN RAT NEOCORTICAL NEURONS MAINTAINED "IN VITRO". G.G.C.Hwa* & M.Avoli (SPON: D.Baxter). MNI, McGill Univ., Montréal, Qué., Canada.

The role played by NMDA receptors in experimental epilepsy has been well documented. Here, we used intracellular recordings to study the effects of NMDA receptor antagonists on BMI (50μM)-induced bursts generated by rat neocortical neurons. The latency of the burst was inversely related to the stimulus strength and the burst duration decreased upon membrane hyperpolarization. Bath application of competitive (CPP) and non-competitive (MK-801) NMDA antagonists increased the burst latency while changing the burst induced by stimuli at threshold into a biphasic EPSP. In addition both NMDA antagonists reduced the late phase of the burst evoked by higher strength stimuli. The increase in burst latency and complete blockade at threshold intensity may reflect an action exerted by NMDA antagonists upon recurrent excitatory mechanisms. Furthermore, since the BMI bursts evoked by suprathreshold stimuli were only reduced, our data indicate that non-NMDA receptors might contribute to this type of experimental epileptiform activity.

345.3

CGP 37849 / CGP 39551: COMPETITIVE NMDA RECEPTOR ANTAGONISTS WITH POTENT ORAL ANTICONVULSANT ACTIVITY. M. Schmutz*, K. Klebs*, H.-R. Olpe*, G.E. Fagg, H. Allgeier*, R. Heckendorn*, C. Angst*, D. Brundish* and J.G. Dingwall* (Spon.: W.B. Adams), Research & Development Dept., Pharmaceuticals Division, and Central Research Labs., CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland.

Evidence has accumulated that antagonists at the N-methyl-D-aspartate (NMDA) sub-type of excitatory amino acid receptor may find value for the treatment of epilepsy and ischaemic neurodegeneration. However, no competitive NMDA receptor antagonists with oral anticonvulsant properties have yet been reported.

CGP 37849 (D,L-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid) and its ethyl ester CGP 39551 selectively inhibited ^{3}H-CPP binding with Ki values of 35 and 310 nM, respectively (for details see G.E. Fagg et al., this meeting), and exhibited potent oral anticonvulsant activity. They blocked electroshock-induced seizures in rodents with ED_{50}'s of 4-21 mg/kg po and 0.4-6 mg/kg ip and iv. The duration of action (plateau effect) exceeded 8 hr in the case of CGP 37849 and 24 hr for CGP 39551. CGP 39551 also delayed the development of overt motor seizures in rat kindling studies at 10 mg/kg po and above, whereas CGP 37849 was not effective under these conditions. The anticonvulsant activity of both compounds is likely to be mediated via blockade of NMDA receptors since orally-effective doses selectively suppressed NMDA-evoked neuronal firing in the rat hippocampus *in vivo*, but had no effect on quisqualate- or kainate-evoked responses.

CGP 37849 and CGP 39551 are the first competitive NMDA receptor antagonists with significant oral activity and, as such, are potential candidates for novel antiepileptic therapy in man.

345.4

INTERACTION BETWEEN N-METHYL-D-ASPARTATE (NMDA) AND BICUCULLINE METHIODIDE (BIC) IN VIVO IN MICE. I.M. Kapetanovic, C. Gennings*, C.D. Torchin* and H.J. Kupferberg*. Epilepsy Branch, NINCDS, NIH, Bethesda, MD 20892 and Dept. of Biostatistics, MCV, Virginia Commonwealth Univ., Richmond, VA 23298.

The balance between inhibitory and excitatory neurotransmission may be important in the pathogenesis and potential treatment of epilepsy. NMDA, agonist of excitatory glutamate receptors and BIC, antagonist of inhibitory GABA-A receptors are commonly used convulsants in experimental animal models of epilepsy. This study examined if the effects of convulsant agents which act by different mechanisms are additive, synergistic or antagonistic. NMDA and BIC were administered i.c.v., individually or as a combination, in saline to male CR:NGP(S) mice. The measured response was at least 5 sec of clonic activity during the 30 min observation period after the administration of NMDA and/or BIC. CD50 values (and 95% confidence intervals) for NMDA and BIC were estimated as 0.405 (0.386,0.425) and 0.194 (0.185,0.203) nmol, respectively. The slopes of dose response curves for NMDA and BIC alone were not parallel. The probability of clonic convulsion was modeled as a function of NMDA and BIC using the logistic regression. Results showed that the logistic model described satisfactorily the experimental data and a significant antagonistic interaction between NMDA and BIC was evident.

345.5

CONVULSANT ACTIONS OF PUTATIVE ENDOGENOUS LIGANDS FOR EXCITATORY AMINO ACID RECEPTORS UPON FOCAL INJECTION IN AREA TEMPESTAS. H. Zrebeet* and K. Gale (SPON: D. Stoff). Department of Pharmacology, Georgetown University Medical Center, Washington, DC 20007.

Quinolinic acid (QUIN), N-acetyl-aspartylglutamate (NAAG), and l-homocysteic acid (HCA) have been proposed as endogenous ligands for NMDA-preferring receptors. Since activation of NMDA receptors is critical for evoking seizures from area tempestas (AT), an epileptogenic site in the deep prepiriform cortex, we examined the effect of these three agents upon unilateral microinjection into AT in awake, freely moving rats.

QUIN (50 nmol) and HCA (160 nmol) consistently evoked bilateral clonic seizures with rearing and often falling (scores 4 or 5). These seizures started within a few minutes following the microinjection and recurred repeatedly over a 45 min period. The convulsive behavior was similar to that observed with intra-AT application of kainic acid (100 pmol), NMDA (10 nmol), aspartate (1.0 umol) and glutamate (1.0 umol). In contrast, NAAG did not evoke convulsive behavior when applied to AT in amounts up to 164 nmol; nor did this dose of NAAG modify the convulsant action of bicuculline (50 pmol) applied to AT 5 min later.

Our data indicate that QUIN and HCA, but not NAAG, produce a functional effect in AT comparable to that produced by aspartate and glutamate, but with a greater potency. As has been shown for aspartate and glutamate in AT, we expect the convulsant effects of QUIN and HCA in AT to be susceptible to blockade by focally applied NMDA receptor antagonists.

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345.6

ANTICONVULSANT ACTION OF A NONCOMPETITIVE ANTAGONIST OF NMDA RECEPTORS (MK-801) IN THE KINDLING MODEL OF EPILEPSY. K.Morimoto*, K.Sato*, M.Okamoto and S.Otsuki*. Dep. of Neuropsychiatry, Okayama Univ.Med.School, Okayama, Japan.

To investigate the role of NMDA systems in kindling, the effects of systemic injection of MK-801, a novel non-competitive antagonist of NMDA receptors, were examined in kindled rats. The results were; (i) Both the seizure stage and afterdischarge (AD) duration of previously kindled seizures from the amygdala (AM) were potently suppressed following MK-801 injection (1-4 mg/kg) in a dose-dependent manner. The maximum effects were found between 2 and 4 hours after the injection. (ii) The MK-801 also showed significant anticonvulsant actions on kindled seizures from the frontal cortex and the dorsal and ventral hippocampus, while the efficacy differed between these kindled sites. (iii) Daily treatment of MK-801 (0.25 and 1 mg/kg) prior to each electrical stimulation of the AM significantly retarded kindling development. During drug sessions of the 1 mg/kg MK-801 for 19 days, all rats showed only partial seizures and the growth of ADs was strongly prevented. (iv) Pretreatment with reserpine did not antagonize the effects of MK-801 on AM kindled seizures. These results indicate that MK-801 has potent anticonvulsant actions on kindled seizures from both limbic and cortical foci, and that NMDA systems may play a critical role in the seizure-triggering mechanism of kindling.

345.7

NMDA RECEPTOR ANTAGONISTS HAVE ANTIEPILEPTOGENIC, BUT NOT ANTICONVULSANT, ACTION AGAINST IN VITRO "KINDLED" ELECTROGRAPHIC SEIZURES S. Stasheff, W.A. Wilson, S. Swartzwelder, S. Clark, and W. Anderson; Departments of Pharmacology, Medicine, and Psychology, Duke University Medical Center and the Veterans Administration Medical Center, Durham, NC

We have developed an in vitro model of epileptogenesis in the rat hippocampal slice which produces seizure-like electrographic discharges in response to kindling-like stimulations. In slices from 24-31 day-old rats, stimulus trains (60 Hz, 2 sec, twice the intensity that evokes the maximum orthodromic population spike) were delivered every ten minutes to s. radiatum of CA3. With repeated stimulations, the severity of afterdischarges (AD) recorded in s. pyramidale of CA1 and CA3 increased, until they stabilized as electrographic seizures (EGSs) with tonic and clonic components and constant durations.

The competitive NMDA receptor antagonist APV and the noncompetitive channel blocker MK801 prevented the genesis of this epileptiform activity. Slices were bathed in 20-100 uM D-APV or 10 uM MK801 prior to and during the presentation of ten stimulus trains. During this period there was no progression of AD toward characteristic EGS activity. Stimulations were then continued in artificial CSF without APV or MK801. Under these conditions, the AD progressed to yield EGSs. Finally, the antagonist was reapplied once the EGSs were established and stable. Neither APV nor MK801 had a significant suppressive effect on these established seizures.

We conclude that, in this model, the suppression of NMDA receptor activity or the opening of the associated channels is profoundly antiepileptogenic. However, once the epileptiform activity is established, NMDA antagonists have little suppressive effect. These results stand in contrast to the effects of NMDA antagonists in suppressing seizure-like activity in several other in vitro models (high K+, low Mg+), but are consistent with their effects on kindling in vivo.

345.8

SYNERGISTIC ANTICONVULSANT ACTION OF NIMODIPINE AND MK-801 IN MICE ADMINISTERED PENTYLENETETRAZOL. G.T. Bolger and S.K. O'Neill*. Division of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland A1B 2V6

Both NMDA-receptor antagonists and nimodipine have been shown to possess anticonvulsant activity in mice administered the cortical convulsant pentylenetetrazol (PTZ). We have investigated the effects of i.p. administered nimodipine and the potent NMDA antagonist MK-801, either alone or in combination, on PTZ (85 mg/kg i.p.) convulsions in male CD-1 mice. PTZ produced severe clonic convulsions and posture loss in 85% of the mice (mean onset 71 $\pm$ 3 sec; mortality 23% following full tonic-clonic seizures). Nimodipine (20 mg/kg) increased the mortality (30%-50%) from PTZ convulsions. In contrast, 10 and 20 mg/kg nimodipine significantly increased (~ two fold) clonic convulsion onset time. MK-801 (0.1 and 0.5 mg/kg) neither altered the onset time nor the number of animals experiencing clonic convulsions, but did prevent death due to PTZ convulsions. Combinations of nimodipine and MK-801 at doses as low as 2 mg/kg and 0.5 mg/kg respectively, resulted in an increased onset (~ three fold) and a reduction (50%) in both the number of animals experiencing clonic PTZ convulsions and the severity of the convulsions. Furthermore, death due to PTZ convulsions was prevented at all dose combinations of nimodipine and MK-801 investigated. These results suggest that the combination of MK-801 and nimodipine may provide a safe and beneficial adjunct therapy for epilepsy.

(Supported by the MRC and the Faculty of Medicine, MUN).

345.9

GLYCINE POTENTIATES PHENOBARBITAL, CARBAMAZEPINE AND MK-801 IN MAXIMAL ELECTROSHOCK SEIZURES. S.L. Peterson, J.T. Trzeciakowski*, L.E. Boehnke*, and R.N. Riegel*. Dept. of Medical Pharmacology and Toxicology, Texas A & M University, College Station, Texas 77843.

The purpose of this study was to evaluate the ability of glycine to enhance the effects of clinically effective anticonvulsants in a standardized model of experimental epilepsy in rats.

Maximal electroshock seizures were induced by passing a 60 Hz, 150 mA and 0.2 sec duration current through saline soaked corneal electrodes. Seizure severity was quanitified by extension/flexion ratios. Statistical significance between anticonvulsant dose-response curves with and without glycine were determined by nonlinear regression analysis.

Glycine (30 or 40 mmol/kg, p.o.) administered 1, 2, 4 or 8 hours before the seizure test had no significant effect on seizure response. However, 40 mmol/kg glycine (p.o., 4 hours before seizure test) significantly reduced the ED50 of phenobarbital (13.2 to 10.7 mg/kg, i.p.), carbamazepine (10.1 to 6.8 mg/kg, i.p.) and MK-801 (7.0 to 3.7 mg/kg, i.p.). Glycine did not significantly affect the ED50 of divalproate or phenytoin.

Glycine potentiation of MK-801 suggests involvement of excitatory amino acid receptors in the mechanism of action of phenobarbital and carbamazepine in maximal electroshock seizures. (Supported by NIH Grant 24566)

345.10

KINDLING DEPRESSES MAGNESIUM REGULATION OF DEPOLARIZING RESPONSES TO AMINO ACID EXCITANTS. D. Martin, M.A. Bowe, J.O. McNamara and J.V. Nadler. Depts. Pharmacology and Medicine (Neurology), Duke Univ. Med. Ctr., Durham, NC 27710.

Previous reports have suggested that enhancement of NMDA receptor function may, at least in part, underlie the kindling phenomenon (Mody, I. and Heinemann, U., Nature, 326, 701 (1987); Morrisett et al., Soc. Neurosci. Abstr., 13, 946 (1987)). This idea was investigated with use of a grease-gap preparation for assaying the depolarizing responses of CA1 hippocampal pyramidal cells to amino acid excitants (Bowe et al., this volume).

Adult male rats were stimulated in the angular bundle until they experienced at least 6 class 4-5 seizures of which the last 3 were consecutive. CA1-subiculum slices were prepared from these animals either 24 h or 30-50 d after the last kindled seizure. A grease barrier was placed at the CA1-subicular border and each hippocampal region was independently superfused. Area CA1 was exposed to 5-6 compartment volumes of each excitant concentration and depolarizing responses of pyramidal cells were recorded relative to their axons in the subiculum.

When tested in the presence of 1 mM Mg^{2+} 30-50 d after the last kindled seizure, CA1 pyramidal cells from kindled rats were more sensitive to NMDA, AMPA and L-glutamate than pyramidal cells from implanted, unstimulated controls. The EC_{50} for NMDA dropped from 18.5 uM to 10.8 uM. When tested in the absence of Mg^{2+}, however, none of the concentration-response curves was affected by kindling. Hence the dose ratio for 1 mM Mg^{2+} against NMDA declined from 3.8 to 2.3. The small depressant effects of Mg^{2+} on responses to AMPA and L-glutamate were absent in slices from kindled rats. None of these changes was detected 24 h after the last kindled seizure.

These findings imply that kindling is associated with a long-lasting reduction in the ability of Mg^{2+} to regulate NMDA and quisqualate receptor function. Such a change could well underlie maintenance of the kindled state. (Supported by NIH grants NS 17771 and NS 16064.)

345.11

REGIONALLY-SPECIFIC REDUCTIONS IN KAINATE AND NMDA RECEPTOR BINDING AFTER ANGULAR BUNDLE KINDLING. M.M. Okazaki, J.O. McNamara and J.V. Nadler. Depts. Pharmacology and Medicine (Neurology), Duke Univ. Med. Ctr., Durham, NC 27710.

Previous studies have suggested that enhancement of excitatory amino acid receptor function plays a role in the kindling phenomenon. We therefore quantitated radioligand binding to kainate and NMDA receptors in brain regions of kindled rats with use of autoradiographic techniques.

Adult male rats were stimulated in the angular bundle until they experienced at least 6 class 4-5 seizures of which the last 3 were consecutive. Control animals were implanted, but not stimulated. Binding experiments were carried out on slide-mounted brain sections prepared either 24 h or 28 d after the last kindled seizure or 24 h after a single evoked afterdischarge. Kainate receptors were labeled with [^{3}H]kainate and NMDA receptors were labeled with L-[^{3}H]glutamate under conditions specific for its binding to this receptor subtype.

Kainate receptor binding had declined by 24-30% in stratum lucidum of hippocampal area CA3 and by 12-16% in the inner third of the dentate molecular layer when tested 24 h after the last kindled seizure. These effects were not detected either 28 d after the last kindled seizure or 24 h after a single evoked afterdischarge. These findings suggest that depression of kainate receptor binding represents one of many transient compensatory responses to repeated seizures and is not associated with maintenance of the kindled state.

NMDA receptor binding had declined by about 10% in stratum radiatum of the rostral hippocampal area CA1, in the rostral part of motor cortex and in layers I-IV of somatosensory cortex when tested 28 d after the last kindled seizure. No significant changes were detected in any other brain region nor in any region examined 24 h after the last kindled seizure. These findings suggest that the enhanced NMDA receptor function in kindled rats cannot be explained by an increased expression of NMDA receptors. Rather, kindling leads to a down regulation of NMDA receptor binding in selected brain regions. (Supported by NIH grants NS 16064 and NS 17771.)

345.12

DEXTROMETHORPHAN (DM) BINDING SITES: SPECIES DIFFERENCES OF ALLOSTERIC MODULATION. M. Klein*, J.J. Paturzo* and J.M. Musacchio. Dept. Pharmacology, New York Univ. Med. Ctr., New York, NY 10016.

Dextromethorphan (DM), a non-narcotic antitussive, binds in the guinea pig and rat CNS to high and low affinity sites. In the mouse, DM binds to a single high-affinity site, but two different sites can be differentiated by competing drugs. Caramiphen (CAR) and carbetapentane (CRB) displace [^{3}H]DM in the low nM range in all three species, and DM, CAR and CRB protect rats and mice against maximal electroshock seizures. Besides, DM and CRB potentiate the effect of phenytoin (PHT) (Tortella and Musacchio, Brain Res., 383:629, 1986). These reports suggested that DM sites are involved in the mediation of the anticonvulsant activity of several drugs. However, there are important species differences: in the guinea pig, 100 µM PHT and 10 µM ropizine (at pH 7.4) increase 4 fold the affinity of DM binding without changing the Bmax. By contrast, the allosteric effects are very small (pH 8.4) or undetectable (pH 7.4) in the mouse and rat brain. These results suggest that DM high affinity binding sites are part of a macromolecular complex which in some species includes allosteric modulator sites. Supported in part by USPHS grants DA-02013, MH-29591, MH-17785 and NS-23926.

345.13

DILTIAZEM ENHANCES AND FLUNARIZINE SUPPRESSES NIMODIPINE'S ANTIEPILEPTIC ACTIONS: A REFLECTION OF ALLOSTERIC BINDING INTERACTIONS AT THE DIHYDROPYRIDINE BINDING SITE? M.A. Moron, T.L. Yaksh and C.W. Stevens. Neurosurg. Res. Lab., Mayo Clinic, Rochester, MN 55905.

The dihydropyridine (DHP) calcium channel antagonist (CCA), nimodipine (NM) has antiepileptic and anticonvulsive properties that are thought to be mediated through neuronal calcium channel blockade. The DHP binding site can be positively and negatively allosterically regulated by the benzothiazepines and phenylalkylamines/piperazines, respectively. In this investigation we studied this binding interaction at the physiologic level by examining the effects of diltiazem (DLT, a benzothiazepine) and flunarizine (FLZ, a piperazine) on the antiepileptic activity of NM. Metrazol (MTZ, 30 mg/kg IP) is used to induce seizures in awake rats with chronically implanted EEG electrodes. CCAs are administered intracerebroventricularly 30 min after MTZ at 3 doses (30, 100 and 300 µg) given at 15-min intervals. DLT and FLZ alone lacked antiseizure properties. The calculated ED_{50} values for NM were: NM alone = 135.3 ± 17.2 µg; NM + DIL (100 µg) = 83.8 ± 24.2 µg. NM + FLZ (10 µg) completely suppressed NM's antiepileptic activity. These findings may reflect the interaction observed among these agents at binding sites associated with the calcium channel and supports the idea that the DHPs mediate their antiseizure actions through neuronal calcium channel antagonism. (Supported by grant NS24329.)

345.14

CARBAMAZEPINE SUPPRESSES ZERO-MAGNESIUM SEIZURES IN RAT HIPPOCAMPUS-ENTORHINAL CORTEX BRAIN SLICES. S. Clark, W.A. Wilson and A.C. Bragdon. Depts. of Pharmacology and Medicine (Neurology), Duke University and Veterans Administration Medical Centers, Durham, N.C. 27710.

Seizure activity can be studied in vitro using brain slices containing hippocampus (HC) and entorhinal cortex (EC) bathed in ACSF containing no added Mg^{++} (0 Mg^{++}). Seizures can be studied for long periods if a low concentration (0.5-5 uM) of baclofen is included in the ACSF. We have employed this model to study the actions of anticonvulsant drugs.

Slices were prepared with EC and HC connections left intact. Pairs of stimulating and recording electrodes were positioned to detect seizures in area CA3 of the HC and the ERC. To study seizure suppression, slices were exposed to 0 Mg^{++}/baclofen ASCF until stable seizure activity occurred, and then the carbamazepine was added.

Carbamazepine, at clinically relevant concentrations (2-12 uM) suppressed spontaneous seizures, increased the threshold required to trigger seizures, and shortened the duration of the seizures.

Supported by NIH grant NS 17771 and the Veterans Administration.

345.15

EFFECTS INDUCED BY THE ANTIEPILEPTIC DRUG VALPROIC ACID (VPA) UPON LOW Mg EPILEPTIFORM BURSTS. V.Tancredi, A.Siniscalchi* and M. Avoli. Dip.Med.Sper., Univ.di Roma "Tor Vergata", Rome, Italy & MNI, McGill Univ. Montréal, Qué. Canada.

Spontaneous epileptiform bursts were recorded in the CA1 subfield of rat hippocampal slices perfused with Mg-free medium. These epileptiform events occurred regularly at a frequency of 0.5-0.05Hz, each lasting 50-500ms. Addition of 2mM VPA evoked a decrease of the frequency of occurence of the epileptiform bursts and a 100-200% increase in duration of each event. In addition prolonged shifts (duration 8-20s) could appear at this stage. These changes were eventually followed by the complete disappearance of paroxysmal activity. A full recovery of the low Mg bursts was observed following VPA wash. Lower doses of VPA did not affect the occurrence or the shape of the low Mg epileptiform bursts. Furthermore in the presence of bicuculline, VPA (2mM) failed to reproduce the effects described above. These results demonstrate an action of VPA upon the low Mg epileptiform activity although the doses required for blocking these bursts appear larger than those used in other "in vitro" models of epilepsy.

345.16

IN VITRO AND IN VIVO ELECTROPHYSIOLOGICAL EFFECTS OF THE ANTICONVULSANT GABAPENTIN. C.P. Taylor, D.M. Rock, R.J. Weinkauf* and A.H. Ganong. Parke-Davis Pharm. Res., Ann Arbor, MI 48105 and Div. Neurosci., Beckman Inst. City of Hope, Duarte, CA 91010.

Gabapentin (1-(aminomethyl)cyclohexaneacetic acid) is a new anticonvulsant currently in clinical trials. Gabapentin has a novel profile of activity against experimental seizures in animals, so electrophysiological experiments were done to investigate cellular mechanisms.

In intracellular recordings from cultured mouse spinal cord neurons, gabapentin (175 μM) had no effect on sustained repetitive action potentials, spontaneous synaptic activity, or iontophoretic GABA or glutamate responses. In extracellular recordings from hippocampal slices, gabapentin (100 μM) had no effect on induction of long-term potentiation. In extracellular recordings from the hippocampal CA1 area of rats in vivo, gabapentin (≥ 1 mg/kg IP) caused a dose-dependent decrease in inhibition measured by paired-pulse stimulation. The cellular mechanism of gabapentin's action on paired-pulse inhibition is not known.

These results indicate that at relevant concentrations gabapentin does not block sodium-dependent action potentials like phenytoin, carbamazepine, or valproate. Gabapentin does not alter long-term potentiation like antagonists of the NMDA glutamate receptor subtype, but has effects similar to phenytoin on paired-pulse inhibition in vivo.

345.17

LACK OF EFFECT OF ASPARTAME ON KINDLING, ELECTROCONVULSIVE SHOCK (ECS) AND METRAZOL-INDUCED SEIZURES IN RATS. L. Thai*, H.A. Tilson, D. Zhao, T. Sobotka[a], and J.S. Hong. LMIN, NIEHS/NIH, P.O. Box 12233, Research Triangle Park, NC 27709 and [a]FDA/CFSAN, Washington, DC 20204.

Kindling was used as a model to examine the effect of aspartame on the seizure threshold. Male, Fischer rats were implanted unilaterally with bipolar electrodes to the right deep prepyriform cortex and were stimulated until 3 consecutive stage 5 convulsions were reached. If aspartame (1 g/kg, p.o.) was given 2 hrs prior to the first stimulation, which was given hourly, followed by a second dose 6 hrs later, the rate of kindling was not affected. If rats were dosed for 14 days (1 g/kg twice daily) and stimulations given hourly on the next day, aspartame had no effect. Aspartame also had no effect on the rate of kindling if rats were dosed twice daily (1 g/kg) and stimulated 2 hrs after each dose. In addition, pretreatment with aspartame (1 g/kg) 6 hr prior to ECS had no effect on the seizure threshold. Similar pretreatment with aspartame had no effect on either the seizure threshold or seizure duration in metrazol-induced convulsions. In conclusion, aspartame failed to show a proconvulsant effect in these three seizure models.

345.18

PRECLINICAL PROFILE OF ISOMERS OF THE ANTICONVULSANT, PR 934-423. G.E. Garske*, G.C. Palmer, M.L. Stagnitto*, J.J. Napier*, R.J. Gentile* and R.C. Griffith* (SPON: C.N. Latimer). Pennwalt Corporation, Pharmaceutical Division, Rochester, NY 14623.

PR 934-423 [(±)-2-amino-N-(1-methyl-1,2-diphenylethyl) acetamide·HCl] has been shown to protect rodents against maximal electroshock seizures (MES). The favorable oral efficacy/safety ratios and low toxicity led to Phase I of clinical testing. Evaluation of the isomers of PR 934-423 yielded the following results (oral doses in mg/kg)(results with the racemate are presented for comparison): ED50 MES mice, rats: PR1032-644(+) 76,33; PR 1032-646(-) 45, 20; & PR 934-423(±) 52, 19.5. Onset of MES protection at the ED98 was 30 min (all compounds) and the duration was 4 hrs for the isomers and 3 hrs for the racemate in mice while in rats these time courses were 30 min and 8 hrs. Values for neurotoxicity (TD50-inverted screen) and safety (LD50) in mice were: 647 and >1,000 for PR 1032-644(+); 598 and 723 for PR 1032-646(-); and 396 and 877 for PR 934-423(±). The resulting therapeutic indices (TD50/ED50) and safety margins (LD50/ED50) were: 8.5 and >13.2; 13.2 and 16.1; and 7.6 and 16.9 for the (+), (-), and racemate respectively. Subchronic dosing of the ED98 to mice resulted in a twofold increase in the ED50 for protection against MES. The findings indicate that in mice the (-) isomer is more potent, while in both species the (+) isomer is weaker. In addition, tolerance to MES protection occurs in mice with the racemate, as well as with the two isomers.

345.19

PR 1013-708, A POTENT ANTICONVULSANT AGAINST MAXIMAL ELECTROSHOCK SEIZURES (MES) IN RODENTS. M.L. Stagnitto*, G.C. Palmer, G.E. Garske*, J.J. Napier*, S.A. McCreedy*, R. C. Griffith* and J.C. Blosser. Pennwalt Corporation, Pharmaceutical Division, Rochester, NY 14623.

PR 1013-708 [2-amino-N-(1,2-diphenylethyl) acetamide·HCl] possesses a high degree of oral potency for protection of rodents against MES (all doses expressed as mg/kg)(ED50 for mice = 11.7, ED50 for rats = 15.5). The time course for protection reveals a 30 min onset with a duration of action of 4 hrs in mice (at the ED98) and greater than 8 hrs in the rat (at 3 x ED50). The convulsive dose 50 (CD50) is 502 in mice and 316 in rats. The toxic dose 50 (TD50) for mice using the inverted screen test is 236 and the LD50 values are 611 for mice and 421 for rats. Efficacy/safety ratios are calculated as: 1) therapeutic index (TD50/ED50), mice = 20; 2) convulsive index (CD50/ED50), mice = 43, rats = 16.2; and 3) safety margin (LD50/ED50), mice = 52, rats = 27. No tolerance to hexobarbital-induced sleep time or anti-MES actions is observed following subchronic administration of PR 1013-708 to rats or mice, respectively. PR 1013-708 is ineffective against seizures elicited by metrazol, bicuculline, strychnine or picrotoxin. No efficacy for binding of PR 1013-708 to receptors associated with GABAa, glutamate, benzodiazepine, adenosine or acetyl choline (muscarinic) is observed using synaptosomal preparations from rat brain. PR 1013-708 appears to be specific for protection against MES and would be predicted to be useful for generalized tonic/clonic seizures.

346.1

ASTROCYTE SUPPORTED NEURITE OUTGROWTH AND NEURONAL ADHESION IS REDUCED AS THE ASTROCYTE MATURES. G.M. Smith*, J. Rudge*, J. Silver and R.H. Miller* (SPON: Marcus Singer). Neuroscience Center, Case Western Reserve Univ., Sch. of Med., Cleveland, Ohio 44106.

Astrocytes have been proposed to support axon outgrowth both in vivo and in vitro. Transplant experiments indicate that astrocyte maturation either in vivo or in vitro results in a decrease in axonal outgrowth. In this study we have examined neurite outgrowth and neuronal adhesion on immature and mature astrocytes in vitro. Astrocytes were purified from newborn rat forebrains to >95% purity by differential adhesion and allowed to mature in vitro (immature <4days, mature >28days). Immature or mature astrocytes were plated onto poly-l-lysine coated coverslips at a density of 60,000 cells to form a monolayer and maintained 24 hours before the addition of neurons. Neurons were isolated from embryonic day 18 rat forebrains and seeded at a density of 5,000 neurons/coverslip to compare neurite outgrowth. Neurites were labelled with tetanus toxin 16, 28, and 40 hours after plating and their length determined. The rate of neurite outgrowth appeared linear on both immature and mature astrocytes. However, neurites were consistently 30-35% longer on immature astrocytes. For short term adhesion experiments neurons were plated at a density of $5x10^5$ neurons/well in either the presence or absence of Ca^{++} (1mM EDTA) and the number of bound cells determined. Neuron adhesion was 4 fold greater to immature astrocytes than mature astrocytes. In the absence of Ca^{++} neuron binding was substantially reduced to mature but, not to immature astrocytes. These results indicate that the capacity of astrocytes to support neurite outgrowth and neuronal adhesion is reduced as the astrocyte matures. (Supported by APA grant RC 88-02; NIH grant NS 25597-07)

346.2

CHARACTERIZATION OF A FACTOR REGULATING NEURON AND NEURITE EXCLUSION IN FRONTAL CORTEX CULTURES. R.D. Todd and P.A. Bauer (SPON: S.B. Guze). Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110

Primary cultures of rat fetal, frontal cortex contain A2B5$^+$, GFAP$^-$, galC$^-$, immobile cells which do not support the movement of neurons or the growth of neurites across their surfaces (R. D. Todd, Soc. Neurosci. Absts. 13:257). The resulting neurite exclusion areas (NEAs) are common in cultures of E16.5 - E17.5 cortex but are rare at earlier and later times. The cells which form the NEAs die after five to seven days in vitro though the cultures as a whole are viable for at least two weeks.

To determine the mechanism of control of the expression of NEAs, we have co-cultured dissociated frontal cortex cells from different aged fetuses and have grown E17 cells in media conditioned by other age cells. The average number of NEAs present in co-cultures of E16 and E17 cells was the arithmetic mean of individual cultures. Culture of E17 cells in media conditioned by E16 cells had no effect on the number of NEAs. In contrast, co-cultured of E17 cells with E19 cells resulted in a decrease in the number of NEAs to that of E19 cells alone. Culture of E17 cells in E19 conditioned media also decreased the number of NEAs to the E19 level. These results suggested that the decrease in NEAs with increasing age is due to an active soluble factor expressed later in development. Heating of E19 conditioned media at 65°C for 15 minutes destroyed this activity. HPLC of E19 conditioned media demonstrated a high apparent molecular weight (~500,000 dalton) fraction of activity which was sensitive to protease K.

346.3

TARGET-SEEKING BEHAVIOR OF EARLY GROWING AXONS FROM RETINAL GRAFTS IN MAMMALIAN MIDBRAIN. M.H.Hankin and R.D. Lund. Dept. Neurobiol., Anat. & Cell Sci., Sch. of Med., Univ. Pittsburgh, Pittsburgh, PA 15261.

In a previous study (J Comp Neurol **263**: 455 (1987)) we showed that retinae placed on the **surface** of the midbrain preferentially project axons along the surface to reach the superior colliculus (**SC**). In contrast, retinae placed in the dorsal midbrain **parenchyma** project directly through the neuropil to innervate the SC.

Here we characterized the earliest outgrowth from these grafts to determine how retinal axons orient towards their target. Monoclonal antibody (M4/6) and normal fiber stains show that axons emerging either from surface or parenchymal grafts were highly oriented towards the SC from the earliest time they could be detected. The time of initial outgrowth from surface grafts (4d posttransplantation (**PT**)) was earlier than was seen from parenchymal grafts (6d PT). In addition, the rate of outgrowth from regions of the graft closer to the SC appeared to be greater than that seen from more distant regions.

Our observations suggest that a hierarchy of cues is involved in guiding optic axons to their targets. It appears that the surface of the brainstem is the preferred ("outgrowth-promoting") substrate. Outgrowth from parenchymal grafts suggests that the SC itself can directly influence the behavior of optic axons independent of the surface of the brainstem surface, provided the fibers are within about 1500 m. Thus the target may help to define both the polarity of elongation and the specificity of innervation. Supported by NEI grant EY05308.

346.4

PERIPHERAL OUTGROWTH OF SYMPATHETIC PREGANGLIONIC NEURITES FROM AN HETEROTOPIC THORACIC NEURAL TUBE TRANSPLANT. P. Cauwenbergs[1], J. Butler[2]* and E. Cosmos[2], Anat. Dept., CMCC[1]. Toronto, Ont. M4G 3E6 and Neurosci. Dept., McMaster University, HSC[2]., Hamilton, Ont. L8N 3Z5.

Thoracic nerves are distinct from limb-innervating nerves because (1) they do not contribute to plexus formation; (2) they alone conduct sympathetic preganglionic axons to the sympathetic trunk. Motor nerves derived from a thoracic neural tube (NT) transplanted to the brachial region of experimental (Thor-Br) chick embryos, however, form a morphologically normal brachial plexus. This occurs despite the fact that the graft develops structural characteristics of an in situ thoracic NT, notably a sympathetic column of Terni (CT); the latter (CT) is absent within the brachial region of control (Br-Br and unoperated) embryos (JEEM 95:147,1986). To determine the pattern of ectopic CT fibre outgrowth cross-sections of Thor-Br embryos were analysed using urea-silver nitrate stain.Results demonstrated that the pattern of ectopic CT fibre outgrowth in the brachial region of Thor-Br embryos duplicated that of in situ CT fibres in the thoracic region.In Thor-Br embryos, CT fibres coursed ventrolaterally within thoracic NT grafts, entered ventral motor roots and, then,diverged from the peripheral nerve to enter the brachial sympathetic trunk. This pattern was observed from day 6E to day 16E. Currently, the fate of ectopic CT fibres within the periphery is under investigation.(Supported by MDAC and NSERC).

346.5

DENTATE GRANULE NEURONS EXHIBIT ADULT NUMBER OF DENDRITIC SEGMENTS EARLY IN DEVELOPMENT. L. Rihn* and B.J. Claiborne (SPON: D. Armstrong). Division of Life Sciences, University of Texas, San Antonio, TX 78285.

Dendritic branching patterns of granule neurons from young rats were quantified and compared to those of adult granule cells described previously (Claiborne et al., *J. Comp. Neurol.*, in press). Pregnant Sprague-Dawley rats were obtained from Zivic Miller and *in vitro* hippocampal slices were prepared from pups 6 to 8 days of age. Granule neurons in the dorsal blade of the dentate gyrus were filled with horseradish peroxidase and, of six complete fills, three were analyzed in detail. As expected, the width of the dorsal molecular layer (150 um average) and the dendritic length per neuron (2003 um) were much less in the young animals than in the adults (257 um and 3478 um). In contrast, both the number of segments (33) and the transverse spread (316 um) were similar in the young and adult neurons (31 and 347 um). These data suggest that although increases in dendritic length may parallel molecular layer development over the first 3 weeks of life, establishment of adult segment number and transverse dendritic spread occur very early and are not directly correlated with molecular layer maturation.
(Supported in part by NSF grant #BNS 8709366.)

346.6

MORPHOLOGY OF CULTURED SYMPATHETIC NEURONS WITH AND WITHOUT DENDRITES COMPARED TO NEURONS IN VIVO. M.I. Johnson and D. Higgins+. Dept. Anatomy & Pediatrics, Washington Univ. Sch. Med., St. Louis, MO 63110; +Dept. Pharm., SUNY Sch. Med., Buffalo, NY 14214.

Cultured sympathetic neurons from perinatal rat superior cervical ganglion maintained 3-4 wks in a chemically-defined medium (DM) predominantly bear one process with the characteristics of an axon. Serum in the medium or Schwann cells in the culture induce processes with dendritic morphology (Bruckenstein and Higgins, Dev. Biol., 1988). In this study the electron microscopic morphology of cultured neurons bearing only axons or those bearing in addition dendrites is compared to that of embryonic, perinatal, and postnatal neurons in vivo. Somata of unipolar neurons maintained in culture in DM contain free ribosomes surrounding a central collection of organelles, an eccentric nucleus and little rough endoplasmic reticulum (RER). The morphology of the same perinatal neurons at the time of initial dissociation is more complex including well-developed RER. With the addition of Schwann cells, cultured perinatal neurons in DM develop dendrites and the cytoplasm now has RER. However, unlike postnatal neurons in situ, the neurons contain many neurofilaments, often in bundles extending out into dendritic processes. These observations suggest that neurons cultured in DM fail to maintain the level of differentiation attained in vivo prior to dissociation. Induction of dendritic growth, particularly by Schwann cells, results in mature cytoplasmic morphology but with increased neurofilaments. (Supported by NIH NS21771, NS22126.)

346.7

COMPARISON OF NIGROSTRIATAL DOPAMINERGIC AXONS AND RADIAL GLIA IN DEVELOPING RAT BRAIN. C.W. Shults,+# R. Hashimoto,++ R.M. Brady,+* F.M. Gage,# +VA Med. Ctr., San Diego 92161, #Dept. Neurosci., UCSD, La Jolla, CA, 92093 ++Yokohama City Univ., Yokahama, Japan

The factors that control the development of the dopaminergic axons from the substantia nigra to the striatum remain unknown. Radial glia provide guidance for migrating neurons and have been suggested to guide axons during development. In the rat at ages E12, E13, E14, and E16 we compared the paths of the nigrostriatal axons and processes of radial glia using a polyclonal antiserum against tyrosine hydroxylase (TH) and a monoclonal antibody against vimentin respectively. At E12 TH-like material was not detected in the developing rat brain. However, vimentin-like material was widely distributed. At E13 TH-like cells, which later develop into the neurons of the substantia nigra and ventral tegmental nucleus, were clearly apparent at the mesencephalic flexure with projections toward the developing striatum. At E16 the TH-like cells in the mesencephalon had become more numerous and abundant axons projecting to the striatum and a few axons projecting to the developing cortex were clearly apparent. AT E16 TH-like axons emanating from the mesencephalon did not parallel vimentin-like processes. However, more rostrally the path of the developing nigrostriatal TH-like axons was similar, through not entirely overlapping with a group of vimentin-like processes.

346.8

AN ULTRASTRUCTURAL STUDY OF THE EARLIEST INTERACTIONS OF PRIMARY VESTIBULAR AFFERENTS IN THE CHICK EMBRYO MEDULLA. R. Petralia and K.D. Peusner. George Washington Univ. Sch. of Med., Washington, D.C. 20037.

The tangential nucleus (TN) is a primary vestibular nucleus which contains the principal cells (PC) that migrate at 6-6½ days and differentiate between 6½ and 8 days. Although the primary vestibular afferents enter the medulla in fascicles at 3 days, these fascicles delay forming synapses on PC until 7½ days. In fact, the very first synapses are formed at 5 days on the processes of primitive epithelial cells (PEC; PC precursors) by longitudinal fibers (LF) of unknown origins. Therefore, the present objective was to determine what ultrastructural interactions occur in the TN between 2 and 5 days.

Before the 7-8th nerve fibers grow in, the presumptive TN contains processes of PEC separated by empty spaces, or "channels". The LF appear within these channels at about the time of entrance of the 7-8th fibers. From 2 to 4 days, no synapses are apparent in the TN. However, attachment plaques and coated inpockets are present on the membranes of the components of the TN, including the primary vestibular afferents, PEC, and LF. Thus, attachment plaques and coated inpockets predate synaptogenesis in the TN. This data will provide a basis to evaluate experimental studies on TN development in the absence of the primary vestibular afferents. Supported by NIH grant R01 NS18108.

346.9

TRANSIENT GABA-IMMUNOREACTIVITY IN CRANIAL NERVES OF THE CHICK EMBRYO. C. S. Von Bartheld and E. W Rubel. Hearing Development Laboratories RL-30, Univ. of Washington, Seattle, WA 98195.

In addition to its function as a neurotransmitter, γ-aminobutyric acid (GABA) influences a variety of processes in the developing central nervous system (Redburn, D.A., and Schousboe, A., eds., Neurology & Neurobiology 32, 1986). Little is known about the distribution and function of GABA in the developing peripheral nervous system. We have investigated the distribution and time course of GABA immunoreactivity in the cranium of chick embryos from 2 to 16 days of incubation (E2-E16). GABA immunoreactivity occurs transiently in motor portions of all cranial nerves (between E4-E10), in restricted parts of the vestibular ganglion (E4.5-E9), including its central and peripheral processes, and in some early, possibly transient, projections from the brain to the spinal cord. In addition, we observed differential onset of GABA immunoreactivity in hair cells of the vestibular (E7) and cochlear endorgan (E9). Transient GABA follows "pioneer" fiber outgrowth and appears to coincide with the formation of early neuronal contacts. In the vestibular nerve, transient GABA is not restricted to portions of the ganglion thought to be of neural crest, or placodal origin. The timing of transient GABA-immunoreactivity is consistent with a change in neuronal phenotype (loss of GABA expression) as well as an elimination of GABAergic neurons (embryological cell death). Transient GABA-immunoreactivity indicates that GABA is not restricted to circuits which will be GABAergic in the adult. The function(s) of transient GABA-expression are unknown; some lines of evidence suggest that GABA may have neurotrophic functions in developing cranial nerves or their target tissue, and may be involved in the regulation of acetylcholine receptors in the developing neuromuscular junction. (Supported by a Max-Kade Fellowship and NIH grant # NS 24522)

346.10

ELECTRON MICROSCOPIC STUDIES OF TRANSIENTLY EXPRESSED ACETYLCHOLINESTERASE ACTIVITY IN THALAMUS AND CEREBRAL CORTEX OF DEVELOPING RATS. R. T. Robertson, L. Seress and C.E. Ribak. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Previous work from this laboratory has demonstrated transient patterns of acetylcholinesterase (AChE) histochemical staining in layer IV of primary sensory cortical regions of developing rats (Robertson, Neurosci. Lett., 75:259, 1987). The laminar and areal distributions of transiently expressed AChE activity correspond to the terminal fields of specific sensory thalamocortical projections, suggesting that transient AChE may serve as a marker for these thalamocortical neurons. The present studies were undertaken to determine the fine structural location of this AChE activity.

Sprague-Dawley rat pups of 8-12 postnatal days of age were perfused with aldehydes and vibratome sections were processed for AChE histochemistry. Some animals received unilateral lesions of the dorsal thalamus 36-48 hr prior to sacrifice. AChE stained sections containing the lateral or medial geniculate body or primary visual or auditory cortex were processed for electron microscopy (Seress et al., Dev. Brain Res., 36:139, 1987).

AChE histochemical reaction product was found associated with the granular endoplasmic reticulum and nuclear membranes of virtually all large projection type neurons of the lateral and medial geniculate nuclei. Glial cells were unstained. In visual and auditory cortices AChE reaction product was found associated with the plasmalemma of axon terminals, as well as with some stellate neurons. In animals that received thalamic lesions prior to sacrifice, AChE reaction product could be found associated with the plasmalemma of degenerating axon terminals in both visual and auditory cortices. The present results are consistent with previous data indicating that AChE is synthesized transiently by thalamocortical projection neurons during the early postnatal period of development.

Supported by NSF grants BNS 87-08515 and BNS 86-15579 and NIH grants NS-25674 and NS-15669.

346.11

CYTOSKELETAL INVOLVEMENT IN RESEALING OF NEURONAL MEMBRANES AFTER INJURY. X.-Y. Xie and J.N. Barrett. (SPON: D: Puro). Dept. of Physiology & Biophysics, Univ. of Miami School of Medicine, Miami, FL 33101.

Axons of cultured rat septal neurons were transected with a laser at distances of > 100 um from the soma. The transected neurons take up Lucifer Yellow and FITC-dextrans (18 kDa and 70 kDa) before, but not after, membrane resealing. Using dye exclusion as an index of membrane resealing, we found that 75% of the axons resealed within 20 min in control medium containing 2 mM Ca. Colchicine (50 uM) increased resealing to 93%, whereas taxol (20 uM) reduced resealing to 21%. Ca, Sr. and Mn (0.1 mM) enhanced resealing, whereas Mg (2 mM) reduced it. Thus it appears that agents that promote disassembly of microtubules (Ca, colchicine) enhance resealing. whereas agents that oppose disassembly (Mg, taxol) inhibit it. Resealing was reduced by the calmodulin inhibitors trifluoperazine, W7 and troponin I, and by cytochalasin E (4 uM), which inhibits F-actin assembly. These results suggest that the normal mechanism for membrane resealing is Ca-dependent and involves cytoskeletal elements and calmodulin. Surprisingly, there was 100% resealing in low ionic strength solutions (5 meq in isotonic sorbitol or sucrose) lacking divalent cations, indicating that under certain experimental conditions membrane resealing may also be achieved by a Ca-independent pathway. Supported by NIH grant NS 12207.

346.12

SERUM-FREE CELL CULTURE OF POSTNATAL RAT HYPOTHALAMIC NEURONS. K.L. Jorgenson, P.R. MacLeish and D.W. Pfaff. The Rockefeller Univ., New York, NY 10021.

We have developed a low density hypothalamic cell culture system. Medial basal hypothalamus was dissected from 2-5 day old rats, enzymatically dissociated (14 U/ml papain) and plated directly on glass coverslips coated with antibodies to Thy 1, a CNS cell surface protein. This substrate allows better neuronal attachment and survival than poly-lysine. L-15 medium supplemented with 25 mM bicarbonate, 0.3% glucose, 2 ug/ml thyroxine, insulin and transferrin, 5 mM taurine, 0.1 mg/ml gentamycin, 2.5 ug/ml Fungizone, 0.7% Methocel and 5 mg/ml bovine serum albumin was added to the cultures, which were incubated at 37 with 5% CO2. This serum-free medium increased neuronal survival and process outgrowth and decreased astrocyte proliferation (based on immunocytochemical staining for glial fibrillary acidic protein) from that observed with serum. Neurons were identified by their characteristic morphology and staining for neuron-specific enolase. Measurement of primary process outgrowth indicated a rate of about 100 microns/day, for up to 4 days in culture. The majority of neurons were unipolar, 20% and 10% had 2 and 3 primary processes, and fewer than 5% had 4 or more. A range of growth cone morphologies, from flattened membranous veils to bulbous structures, was observed. Somata were 10-20 microns in diameter. This culture system, in which individual cells can be easily studied, is suitable for testing potential growth factors.

346.13

CRUSTACEAN PEPTIDERGIC NEURONS IN PRIMARY CULTURE SHOW IMMEDIATE OUTGROWTH IN DEFINED MEDIA. R. A. Graf, S. Grau*, B. Haylett* and I. Cooke. Békésy Laboratory of Neurobiology and Department of Zoology, University of Hawaii, Honolulu, HI 96822.

Unlike most neurons in primary culture, which require conditioning factors for outgrowth, the secretory neurons of the crab (Cardisoma carnifex) and lobster (Panulirus marginatus) X-organ show immediate, vigorous outgrowth in simple, defined media (e.g. crab saline + glucose, gentamicin, pH 7.6, 25°) when isolated on a variety of substrates (routinely, Primaria). Within 18 h the morphology is established: small cells (<25 um), which include those showing RPCH immunoreactivity, have a sparsely branched major neurite extending >100 um from the axonal stump; some larger cells show similar form, but most produce a broad lamellipodium, often larger in area than the soma. These show immunoreactivity to anti-CHH, anti-MIH or anti-D sera. Growth of veiling cells ceases by 10d, while branching cells continue longer. Additional growth and altered form result from various manipulations such as brief exposure to elevated $[K]_o$ or co-culture with a sinus gland. We suggest that these cells can show immediate outgrowth by utilizing part of the secretory machinery, exocytosis without membrane reuptake, for addition of membrane. In support: a) EM's show microtubules and secretory granules in filopodia; b) growth cones and presumed Golgi are particularly immunoreactive; c) hormonal peptides are released to the media; d) cells exhibit Ca currents (Meyers, et al., this vol.); e) growth, secretion and Ca currents are blocked by Cd. We thank H. Schooneveld and R. Keller for antisera. Supported by NSF BNS84-04459 and NIH NS 15453 to I.C.

PROCESS OUTGROWTH, GROWTH CONES, AND GUIDANCE MECHANISMS VII

347.1

SEX AND THE SINGLE NEURON: GENETIC CONTROL OF SEX SPECIFIC AXONAL PROJECTIONS IN Drosophila SENSORY NEURONS. D.R. Possidente* and R.K. Murphey. Department of Biology, SUNY Albany, NY 12222.

The mechanism by which orderly axonal projections and the corresponding synaptic connections are formed during development remains an important and largely unsolved problem in neurobiology. It may be possible to bring genetic and molecular techniques to bear on this problem by examining the genetic control of axonal growth in Drosophila. We show here that gustatory axons in Drosophila are sexually differentiated and that genes involved in sex determination control the anatomy of these axons. Both males and females possess gustatory receptors on their legs and in females the axonal arborization from these receptors are exclusively unilateral. Males possess significantly more gustatory receptors, and their axonal arborizations are usually bilateral. In diplo-X "males" mutant for the genes tra or Sxl, the gustatory system is transformed toward the male phenotype both in number of receptors and in afferent projections. In order to determine the locus of this effect we examined gynandromorphs and found that the sex of the sensory neuron and apparently not the central nervous system, controls the behavior of the axons. Supported by an NIH Javits Neuroscience Investigator Award to RKM.

347.2

MODALITY SPECIFIC AFFERENT PROJECTIONS IN THE FLIES PHORMIA AND DROSOPHILIA. G.S. Pollack[1], D. Possidente*[2] and R.K. Murphey[2]. [1]Department of Biology, McGill University, Montreal, Québec H3A 1B1 and [2]Neurobiology Research Center, SUNY Albany, Albany, NY 12222.

The legs of flies bear numerous sensory hairs which serve tactile, gustatory, and proprioceptive functions. We examined the afferent projections of these sensilla and found that neurons encoding these three modalities project to distinguishable regions of thoracic neuropile. Proprioceptive neurons are associated with clusters of hairs located near joints (hair plates). Their projections are characteristically more extensive and more dorsal than those of the other two modalities. Tactile receptors are associated both with singly innervated hairs and with multiply innervated hairs, which contain one tactile neuron and four gustatory neurons. Both the tactile and gustatory neurons project to a ventral region of neuropile, but are distinguishable because (1) the gustatory neurons project more medially and posteriorly than the tactile neurons, and (2) the tactile neurons are larger in diameter.

Our findings demonstrate that there is a clear modality specific segregation of axonal arbors in the CNS. We presume that, as in other insect sensory systems, this anatomical specificity is linked to specific synaptic connectivity.

347.3

SPATIALLY RESTRICTED BINDING OF MONOCLONAL ANTIBODIES AGAINST WINGS OF DROSOPHILA MELANOGASTER. P. B. Snyder*, M. A. Murray & J. Palka. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195

Because of the strong evidence that significant guidance information is provided to pioneer sensory axons in the Drosophila wing by the epithelial substrate, we have generated monoclonal antibodies against wings during the axonogenic period. 2G2 and 3C11 stain extracellular material located throughout the wing but concentrated along the veins. This distribution is similar to but not identical with that of a vein-associated antigen we have described previously (Murray, M. A. & Palka, J. Abstr. Soc. Neurosci. 13, 1143, 1987.). 1E1 shows staining exclusively along the third vein (one of two axonal tracts in the wing blade). This pattern is reminiscent of the binding distribution in Drosophila wings of INO, a monoclonal antibody that inhibits neurite outgrowth of cultured mouse neurons (Matthew, W. D. & Patterson, P. H. Cold Spring Harbor Symp. Quant Biol. 48, 625-631, 1983; Blair, S. S. & Palka, J. Abstr. Soc. Neurosci. 13, 6, 1987.). Hence, we currently have a collection of monoclonal antibodies, presumably directed against extracellular matrix components, which show overlapping but distinct distributions. We plan to analyze their binding in other Drosophila tissues and biochemically characterize the corresponding antigens. We also hope to be able to address the question of their possible role in axon growth.

347.4

A MUTATION THAT PERTURBS SELECTIVE SYNAPSES IN THE GIANT FIBER PATHWAY OF DROSOPHILA. F. Katz*, Z. Smith, W. Moats*, and M. Tanouye°. Howard Hughes Med. Inst. and Dept. Biochemistry, UT Southwestern Med. Ctr., Dallas TX, and °Dept. Biology, Cal Tech, Pasadena, CA.

The giant fiber pathway in Drosophila mediates a quick escape response. The giant fiber synapses with the TTM motoneuron (TTMm) innervating the TTM, or jump muscle. The giant fiber also innervates a postsynaptic neuron which then synapses upon the motoneurons for the DLM wing depressor muscle (Wyman et al. in: Neural Mechanisms of Startle Behavior, R.C. Eaton, ed. Plenum, 1984). A mutant was identified on the 3rd chromosome in which the pathway to the DLM is electrophysiologically normal, but the response of the TTM is aberrant, having a delayed latency much like the X-chromosome mutant bendless. Cobalt fills of the giant fiber through the antennal nerve reveal that the giant fiber is morphologically normal. Horseradish peroxidase backfills of the TTMm from the TTM indicate the TTMm is connected to the jump muscle and also appears normal. Physiological experiments are under way to pinpoint the lesion in the pathway from the giant fiber to the jump muscle. The mutation has been mapped to the proximal left portion of the third chromosome.

347.5

INTERACTIONS BETWEEN IDENTIFIED MOTONEURONS DURING PATHWAY SELECTION IN ZEBRAFISH EMBRYOS. S. Pike* and J.S. Eisen. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Each side of every trunk segment in the zebrafish is innervated by 3 identified motoneurons that extend axons out of the spinal cord in a stereotyped temporal and spatial pattern. The growth cones of all of these cells follow a common pathway to a region where they pause before selecting cell-specific pathways that lead to mutually exclusive muscle territories. We tested whether CaP, the first motoneuron to extend a growth cone, had a unique ability to establish the common pathway. Ablation of CaP using laser irradiation did not affect the ability of the growth cones of the other identified motoneurons, MiP and RoP, to leave the spinal cord and extend along the common pathway. We also tested whether interactions among motoneurons were required for cell-specific pathway choice. Following CaP ablation, the MiP and RoP growth cones initially selected their normal, cell-specific pathways. These results suggest that CaP is not necessary to establish the common pathway, and that interactions with CaP are not required for MiP and RoP initially to select their normal, cell-specific pathways. We are currently examining whether interactions among motor growth cones may affect later pathway choices. Supported by the NIH, NSF, Chicago Community Trust and a Patricia Roberts Harris fellowship.

347.6

MESENCHYMAL CELL DEATH DELINEATES AXON PATHWAYS IN THE HINDLIMB AND DOES SO INDEPENDENTLY OF NEURAL INTERACTIONS. S. Schroeter, J. A. Pokrzywinski* and K. W. Tosney, Biology Department, The University of Michigan, Ann Arbor, MI 48109.

We wished to know whether the mesenchyme cell death seen near growth cones in the hindlimb delineates axon pathways and, if so, whether the death was "murder" (required an interaction with growth cones) or "suicide" (independent of neural interactions and thus directly or indirectly characteristic of whatever processes generate the paths). We unilaterally deleted the lumbosacral neural tube and reconstructed the patterns of neurite outgrowth and mesenchyme cell death during the stage when neurites first colonize the thigh. In the control limbs, axonal pathways coincided with sites of cell death. Dying cells were abundant where axons ramified extensively, such as plexus regions and at foci within the muscle masses that correspond to regions where muscle nerves will form. In contrast, dying cells were not seen in adjacent non-pathway regions, such as posterior sclerotome or dorsal and ventral to the region of the plexus in which axons extend only posteriorly. In the experimental limbs, neurite outgrowth was reduced to less than one-tenth of normal (a few neurites were visible with electron microscopy) or to less than one-third of normal, extended less far distally and, in half the cases, motor innervation was completely abolished. Despite the extensive reduction in neurite outgrowth, the distribution of cell death was indistinguishable from the control side. Furthermore, it did not differ significantly in abundance. We conclude that mesenchyme cell death delineates axon pathways remarkably well and does so without an interaction with growth cones. It is an independent characteristic of axonal pathways and may in some way help to guide axons.

Supported by NIH grant NS 21308.

347.7

PEANUT AGGLUTININ (PNA) BINDS TO TISSUES THAT ACT AS BARRIERS TO AXON ADVANCE IN THE CHICK EMBRYO. R.A. Oakley & K.W. Tosney Neuroscience Program & Biol. Dept., Univ. of Michigan, Ann Arbor, MI. 48109

Several tissues in the developing chick embryo can be viewed as barriers to axon advance. These include the pelvic girdle precursor, the posterior sclerotome, and the perinotocordal mesenchyme (ventromedial sclerotome surrounding the notocord). Surgical deletion studies have shown that nerve paths expand spatially when the pelvic girdle or sclerotome are deleted and that motor axons alter their trajectory to avoid the perinotocordal mesenchyme (Tosney & Landmesser, *J. Neurosci.* 4: 2518; Tosney, *Devel. Biol.* in press). The posterior sclerotome is resistant to axon invasion and has been shown to differentially bind PNA (Stern et al., JEEM 91: 209). To determine if the binding of this lectin is typical of barrier tissues, we studied PNA binding in chick embryos in relation to the timing of axon outgrowth.

Stage 18-25 embryos were processed according to Stern et al. Frozen sections (10 μm) were stained with FITC-PNA. We found differential binding of PNA to the pelvic girdle precursor, posterior sclerotome, and to the perinotocordal mesenchyme of both posterior and anterior sclerotome. In anterior half segments, PNA binding was specifically limited to the perinotochordal mesenchyme and did not include the spinal nerve path. PNA binding in the posterior sclerotome extended more laterally, up to the myotome and to the limb base. PNA binding to the pelvic girdle precursor was detected as the earliest growth cones invade the plexus region just medial to it. PNA did not bind to nerve paths including the plexus region and the hiatuses through the girdle which normally transmit axons to the limb. Thus, a PNA binding epitope is common to several barrier tissues and may be involved in constraining the patterns of axon outgrowth. Supported by NIH #NS21308.

347.8

SPECIFICITY OF MIGRATION OF NEURAL CREST CELLS IN THE PERIPHERAL NERVE PATHWAYS OF CHICK EMBRYOS. E. M. Carpenter and M. Hollyday#. Committee on Neurobiology, University of Chicago, Chicago, IL 60637 and #Department of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

Schwann cells, the glia of the peripheral nervous system, are derived from the neural crest. In order to help clarify the role of Schwann cells in limb innervation, we have examined the distribution of neural crest-derived Schwann cells at brachial levels in the chick embryo using the chick-quail chimera technique. Chimeras were prepared by transplantation of a short segment of dorsal neural tube from a quail donor to a chick host at levels of the neural tube destined to give rise to brachial innervation. Embryos were allowed to survive to stages 22-29, then were fixed, sectioned at 4μ, and stained using the Feulgen method of staining to visualize the quail nucleolar marker. The identity of the spinal segment or segments affected by the graft and the identity of the peripheral nerves containing quail cells were determined by reconstruction using the light microscope.

As expected from the results of others, quail cells were observed in the dorsal root ganglia, the sympathetic ganglia, and in the ventral roots of spinal segments affected by the grafts. In addition, quail cells were seen in the spinal nerves and in peripheral nerves innervating the wing. Quail cells were distributed along the entire length of the nerve pathways, with increased concentrations of quail cells present in the plexus region and at nerve branch points. Quail cells were not seen in advance of the growing nerves at any of the stages examined. Quail-derived Schwann cells were distributed in the peripheral nerves in a pattern dependent on the segment or segments affected by the grafts. This pattern closely matches the pattern of innervation established by motor axons emerging from the same segmental level of the spinal cord. These results suggest that Schwann cells accompany emerging nerve fibers from the same segmental level and use these fibers as substrates to guide their migration into the periphery.

Supported by NIH grant NS 25340 to MH. EMC is supported by PHS GM 07839.

347.9

DISAPPEARANCE AND REAPPEARANCE OF FIBRONECTIN ALONG AXONAL PATHWAYS OF THE DEVELOPING PERIPHERAL NERVOUS SYSTEM OF THE CHICK. J.W. Yip and Y.P.L. Yip*. Dept. of Physiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

A molecule that has been shown to modulate morphogenetic movements during gastrulation and mediate the *in vivo* and *in vitro* migration of neural crest cells is the glycoprotein fibronectin. Although fibronectin has also been shown to promote neurite growth *in vitro*, its role in the outgrowth of neurons *in vivo* is less clear. As a first step towards understanding the role of fibronectin in axonal outgrowth *in vivo*, we have examined the relationship of peripheral nerves with fibronectin at different developmental stages of the chick embryo using double immunofluorescent staining. Cryostat sections of embryos from stages 16-44 (2-18 days) were stained with EC/8 antibody against intermediate filaments in neuronal processes and with mono- or polyclonal antibodies against fibronectin. The relationship between motor, sensory and sympathetic neurons with fibronectin was examined from the earliest times they extend processes into the periphery. Prior to axonal outgrowth, fibronectin was widely distributed both within and outside of the presumptive axonal pathways. But soon after the initiation of axonal outgtowth, fibronectin began to disappear along axonal pathways. Thus during the period of active axonal growth, all neural tissues, including neuronal cell bodies and their processes, were marked by the striking absence of fibronectin. Interestingly, fibronectin immunostaining began to reappear along peripheral pathways soon after projection patterns were estahlished and increased in intensity with age. With the exception of their blood vessels, the spinal cord and peripheral ganglia remained devoid of fibronectin.

While the significance of this relationship is not known, these results indicate that growing axons are able to modify their pathways. In modifying the substrate of their pathway, neurons may alter the degree of neuron-substrate or neuron-neuron interaction. Finally, because fibronectin is distributed throughout the mesenchyme during the period of axonal outgrowth, it does not appear to be the molecule required for directing axons to their target region.

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347.10

AXONAL GROWTH CONES IN EMBRYOS WITH REVERSED SPINAL CORD POLARITY. R.H. Nordlander and S.M. Bunt. Dept. Oral Biol., Case Western Reserve Univ., Cleveland, OH 44106 and Dept. Anatomy, Univ. Dundee, Scotland.

Growth cones of developing longitudinal spinal pathways were examined as they approached and entered spinal cord segments that had been rotated 180° rostrocaudally. Five segment long pieces of Xenopus embryonic neural tube were surgically reversed at stages 21-23, 15-20 hrs prior to the arrival of descending supraspinal axons and before growth of ascending fibers from caudal spinal neurons. Growth cones were visualized with HRP applied to the ventral medulla or directly to the spinal cord rostral or caudal to the rotated segment. Specimens were examined for growth cones 6-60 hrs following surgery and after 7 or 13 days for overall fiber distribution.

Many growth cones of descending axons seemed to enter and navigate the transposed segment with ease, though tangles of axons often appeared temporarily at its interface and some fibers spilled out of the prelesion segment onto surrounding tissues. Growth cones of descending pathways made immediate corrections in dorsoventral position in cases of misalignment of the reversed segment.Ascending sensory growth cones seemed to have more difficulty entering the rotated segment and in finding their appropriate position once there. By 7 days specimens looked nearly normal with few axonal tangles and ectopic fibers.

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347.11

PATHWAY FORMATION BY DEVELOPING SPINAL INTERNEURONS FOLLOWING PERTURBATION OF PUTATIVE GUIDANCE CUES. H. Yaginuma*, R.W. Oppenheim and O.E. Usun* (SPON: M.A. Bell). Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

Invertebrate studies and studies of the vertebrate peripheral nervous system have revealed specific axonal guidance mechanisms (e.g., differential adhesion, selective fasciculation). In an attempt to determine whether guidance cues in the vertebrate CNS are similar, we have been examining the trajectories of axons comprising longitudinal intersegmental pathways in the chick embryo. Although a number of different perturbation studies are in progress, we wish to report here the effects of neural tube rotations about the dorsal-ventral (D-V) axis on pathway formation. Three segments of thoracic neural tube from stage 14-15 (day 2) embryos were rotated 90 or 180 degrees. On day 6 (stage 28) HRP was injected rostral to the rotated cord (RC). The results following 90°rotation indicate that upon contacting the rotated cord, labelled axons in both the lateral and ventral marginal zone (MZ) shift position to reach their appropriate location in the rotated MZ, a maneuver that they repeat upon reaching the opposite boundary between rotated and normal spinal cord. Similar shifts in trajectory were not observed to correct for errors in laterality of projections. Preliminary results of 180°rotations indicate that axons fail to correct their trajectories when encountering the RC. That is, axons in the ventral MZ continued to grow straight through the dorsal MZ of the RC. These results suggest that axons of spinal interneurons may utilize specific "short-range" topographic cues for maintaining their longitudinal trajectories. However, in the absence of these cues (e.g., after 180 rotation), axons can continue to extend in a "foreign" environment over relatively long distances. Supported by NSF 8707290.

347.12

EARLY AXONGENESIS AND PATHFINDING IN THE EMBRYONIC MOUSE TRIGEMINAL SYSTEM. D.Y.R. Stainier* and W. Gilbert. Bio Labs, Harvard Univ., Cambr. MA 02138

We are investigating the outgrowth and pathfinding characteristics of specific neurons in the embryonic mouse.

Wholemount preparations of E9 to E12 mouse embryos are being processed immunohistochemically with several mAb's, each with a different specificity. MAb B30 (Stainier et al., Soc. Neurosci. Abstr. 391.13, 1987) stains the surface of mesencephalic trigeminal neurons (mesV) in the CNS as well as other sensory neurons in cranial nerve and dorsal root ganglia. We focus on the trigeminal system and follow its development in both the CNS and the PNS.

MesV neurons are first defined by B30 immunoreactivity at late E9. From their midbrain location, they send axons down to the brainstem where they exit the CNS at the pontine region and mix with stained neurons and axons in the V^{th} (trig.) ganglion. At this stage, these V^{th} ganglion neurons are truly bipolar and they send short processes dorsally towards the pontine region and ventrally towards their peripheral targets. By late E10, three distinct bundles of axons leave the ventral side of the ganglion. This segregation originates within the ganglion itself. Indeed, the mandible innervating neurons form a separate entity at the caudal end of the ganglion. Single axons are also seen leaving the ganglion at other points along its circumference but they quickly join one of the three bundles. By E12 V^{th} ganglion neurons, pseudounipolar at this stage, have reached their target area where their axons branch out heavily.

347.13

RESPONSE OF REGENERATING GOLFISH RETINAL AXONS TO TECTAL CELL MEMBRANES OF ADULT FISH AND EMBRYONIC CHICK

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Retinal axons from embryonic chick (E6) *in vitro* respond to position specific properties of membranes of embryonic tecta (E9 to E13), as shown by Walter et. al.(1987)

Employing their assay we pursued wether regenerating retinal axons from adult goldfish recognize differences between rostral and caudal membranes 1. of adult fish tecta and 2. of embryonic chick tecta. Cell membranes were prepared from either rostral or caudal tectum and arranged in alternating narrow stripes.

1. Fish axons from temporal retina accumulate on membranes derived from their retinotopically related rostral tectum. Nasal axons grow randomly over both types of membranes. Thus, adult fish axons from temporal retina respond to position specific differences of fish tectum in a similar mode as embryonic chick axons do on chick membranes (Walter et. al.,'87).

2. Confronted with tectal membranes from embryonic chick, temporal axons from adult fish accumulate on rostral membranes, as before. Surprisingly, fish nasal axons also choose to grow on rostral membranes of chick.

According to Walter et. al. (1987) temporal chick axons grow on rostral membranes since they avoid an inhibitory component on caudal membranes. We speculate that in fish, both the temporal and nasal axons are sensitive to the chick inhibitor.

347.14

STRUCTURAL ANALYSIS OF A FAMILY OF SENSORY AND GLIAL CELL SPECIFIC 130 KD GLYCOPROTEINS. M. L. Bajt and B. Zipser, Dept. Physiology, Michigan State University, East Lansing, MI 48824.

In the leech nervous system, a group of cell type specific 130 kD glycoproteins differentiates between specific neurons, glial cells, and connective tissue. Four of these 130 kD glycoproteins are neuronal, distinguishing between the full set of sensory afferents and 3 progressively smaller subsets. The fifth and recently isolated sixth are specific for the macroglia enveloping axons and the connective tissue that surrounds the axon tracts, respectively.

The protein cores are being compared by peptide mapping, using limited proteolysis with V8 protease and proteinase K, and analyzed by Western blotting. The two 130 kD proteins expressed by the full set and by the largest subset of sensory afferents are cleaved into identical fragments. The 130 kD proteins expressed by the small sensory subset and by the glial cells are cleaved into fewer of these same molecular weight fragments. Thus, some of the protein cores are homologous, while others are partially homologous.

In lectin affinity chromatography, the 130 kD proteins were previously shown to be mannose rich glycoproteins. Presently, we have deglycosylated the 130 kD proteins on immunoblots using N-glycanase, releasing N-linked oligosaccharides. Lack of binding to deglycosylated proteins demonstrates that the mAb specific for the full set of sensory afferents reacts with a carbohydrate epitope. Each of the 130 kD glycoproteins may have its own unique carbohydrate epitope, since only the mAb specific for the full set of sensory afferents is blocked by preincubation with alpha methyl mannoside. Thus, the same or partially homologous protein cores appear to be differentially glycosylated, thereby generating the different members of the group of 130 kD glycoproteins. Carbohydrate recognition involving the 130 kD glycoproteins may play a role in the formation of the nervous system.

347.15

130 KD LEECH PROTEINS ISOLATED WITH MABS GENERATED AGAINST N-GLYCANASE TREATED IMMUNOBLOT BANDS. I. S. Thorey* and B. Zipser. Dept. Physiology, Michigan State University, East Lansing, MI 48824.

Previously, a group of cell type specific 130 kD glycoproteins has been characterized in the leech nervous system using monoclonal antibodies (mAbs). The antibodies specifically recognize sets and subsets of sensory afferents, axon tract glia or connective tissue. They were generated against fixed CNS tissue or 130 kD gel-bands prepared from CNS tissue. Some of these mAbs were shown to react with N-linked carbohydrate moieties on the 130 kD proteins.

Presently, we have produced a new generation of mAbs by immunizing mice with 130 kD glycoproteins from immunoblots treated with N-glycanase, which cleaves N-linked carbohydrates. In the new fusion, 60 hybridoma clones where isolated, a third of which specifically bind to 130 kD proteins as analyzed on Western blots. On wholemount ganglia, the new mAbs stain components of the central and peripheral axon tracts. On cross sections of whole leech, they also stain the muscles in the body wall. While the new mAbs recognize epitopes shared by more than one cell type, their intensity of staining is generally weaker compared to the previous generation of mAbs. Thus, we might have isolated a new class of 130 kD proteins with lower abundance, and/or the stronger staining exhibited by our previous mAbs may reflect binding to repeated carbohydrate epitopes on the same protein. Conversely, our new mAbs might bind to an epitope on a more ubiquitously distributed conserved core shared by many 130 kD proteins. The core epitope might be masked in some of these 130 kD proteins, creating a lower level of antibody binding. Presently, we are characterizing further our new mAbs to determine their relationship to our first generation of mAbs specific for a group of 130 kD glycoproteins.

347.16

EARLY LABELING OF A MACROGLIAL CELL ANTIBODY IN THE EMBRYONIC LEECH, HAEMOPIS MARMORATA. R.N. Cole*, R. Morell*, and B. Zipser (SPON: S.R. Heisey). Dept. of Physiology, Michigan State Univ., East Lansing, MI. 48824.

We are studying the differentiation of macroglial cells in the developing nervous system of the leech Haemopis marmorata using monoclonal antibodies (mAbs). In the adult, the mAb Laz6-297 binds to a 130 kD surface glycoprotein expressed by macroglial cells associated with the central neuropil, interganglionic connectives, and the ganglionic roots.

Before ganglia and axon tracts are formed (5 days of development at 20C), a novel chain of cells is detected by Laz6-297 in the midline of the embryo. The chain consists of a few cell bodies confined to the posterior region of the germinal plate, with processes projecting anteriorly along the midline to the embryonic mouth. This novel cell type, which is persistent through 8 days of development (20C), demarcates the boundary between the right and left halves of the germinal plate in an arrangement similar to the more numerous midline cells detected in the leech Hirudo medicinalis by the mAb Lan3-2 (McGlade-McCulloh and Zipser, Soc. Neurosci. Abst. 13:1221, 1987).

During axon tract formation, Laz6-297 stains macroglial cells of the connectives, neuropil, commissures, and roots in an anterior-posterior temporal gradient. For example, at 10 days of development (20C) Laz6-297 stains the anterior half of the nerve cord; however, by day 11 more than two-thirds of the nerve cord is labeled. This temporal staining pattern reveals three processes exiting each side of the posterior ganglia and the apparent fusion of the two anterior processes in the anterior ganglia.

The early labeling by Laz6-297 allows us to characterize the ontogeny of glial cells and the nature of glial-neuronal interactions during development. Of particular interest to us are the interactions involving different 130 kD surface glycoproteins which distinguish glia, neurons, and connective tissue during neurogenesis.

347.17

MORPHOLOGY AND SHARED MOLECULAR PROPERTIES OF PROCESSES IN FORMING PERIPHERAL AND CENTRAL AXON TRACTS. N. Moore*, R. Morell*, and B. Zipser . Dept. Physiology, MSU,E. Lansing, MI 48824.(SPON: R.Bernard)

We are studying the formation of central and peripheral axon tracts in the leech using mAb Laz1-1 as a cell type specific marker. Centrally, the earliest processes identified in the future connective belong to the transient bipolar cells which, because of their morphology and time of appearance, were suggested to play a role in the establishment of these longitudinal pathways. Our data on the special characteristics of bipolar cells in head and tail ganglia further support this idea: (1) Bipolar cells in the head ganglia extend their processes rostrally to the edge of the germinal plate and persist late into development, until the tract between the head and supraesophageal ganglion is formed. Their extended survival is in contrast to the gradient of bipolar cell death observed in midbody ganglia. The bipolar cells in the head may persist to help form the axon tract. (2) In tail ganglia, bipolar cells deviate from their usual ipsilateral course and project contralaterally, forming a terminal loop. Thus, bipolar cells may delimit the posterior extent of the connectives. Peripherally, early stained processes belong to sensory afferents which may pioneer these peripheral tracts. Therefore, processes postulated to pioneer central and peripheral axon tracts share the earliest cytoplasmic epitopes detected in primordial ganglia and peripheral neurons.

Ten percent of the central neurons also express Laz1-1 reactive cytoplasmic proteins as they differentiate. These Laz1-1 reactive proteins are only partially Triton X-100 extractable under conditions which do not extract Lan3-8 reactive cytoskeletal proteins. The staining of large cell bodies tends to fade with detergent extraction while the staining of small cell bodies remains strong. We are trying to determine which cytoskeletal associated proteins Laz1-1 may recognize.

347.18

CYTOSKELETAL PROTEINS IN LEECH NEURONS: ACTIN, TUBULIN, NEUROFILAMENT, SPECTRIN AND TALIN. J. W. McRorie III* and B. Zipser (SPON: L. O'Kelly). Dept. of Physiology, Michigan State University, East Lansing, MI 48824

We are studying the cytoskeleton of leech (Hirudo) neurons using mAbs generated against vertebrate cytoskeletal proteins. Each antibody stains dissociated neurons with a distinct pattern and reacts with extracted leech CNS proteins on immunoblots. MAb C4 (generated against 43 kD chicken gizzard actin, Lessard,J., in press) reacts with a 42 kD band. It stains a fibrous meshwork in growth cones and veils, while in neurites it stains both a fibrous meshwork and thicker bundles. MAb 3F3 (generated against rat tubulin, Akeson, pers. com.) reacts with a 54/57 kD doublet and stains a fine fibrous network in veils and in growth cones which have a flattened morphology and numerous filopodia. Rounded neuritic terminals with few/no filopodia have a much greater intensity of fluorescent staining. MAbs 06-68 and 0240 (generated against rat neurofilament, Sternberger , L., PNAS, 80: 6126, 1983) react with major bands at 150 kD and stain fibrous patterns in all processes. Both anti-alpha-spectrin (generated against 180 and 62 kD chicken erythrocyte alpha-spectrin, Koenig, E. and Repasky, E., J. of Neuroscience, 5(3):705-714, 1985) and talin antiserum (Burridge, K., Cancer Review, 4: 18-78, 1986) react with leech proteins on immunoblots and stain dissociated neurons in culture. Talin antiserum stains a coarse, granular pattern with high intensity aggregrates on neuritic processes. We plan to characterize these leech cytoskeletal proteins at the electron microscopic level.

INTERACTIONS BETWEEN NEUROTRANSMITTERS II

348.1

ROLE OF PROSTANOIDS IN THE POTENTIATION BY NORADRENALINE (NA), HISTAMINE (HIS) AND ADENOSINE (AD) OF VIP-STIMULATED cAMP FORMATION IN MOUSE NEOCORTEX. N.C. Schaad*, M. Schorderet[1] and P.J. Magistretti. Département de Pharmacologie, CMU, 1211 Geneva and [1]Ecole de Pharmacie, Lausanne, Switzerland.

We have previously shown that NA, by acting at α_1-adrenergic receptor, potentiates the accumulation of cAMP elicited by VIP in mouse cerebral cortical slices (J. Neurosci. 5, 362-368, 1985). This α_1-receptor-mediated action of NA is antagonized in a concentration-dependent manner (IC_{50} = 3 µM) by indomethacin and diclofenac, two inhibitors of cyclooxygenase, and by mepacrine and p-bromo-phenacylbromide, two inhibitors of phospholipase A_2 (Nature 368, 637-640, 1987). These observations indicate that prostanoids, formed as a result of α_1-receptor occupation, potentiate the accumulation of cAMP elicited by VIP. Among various prostanoids tested, only PGE_2 and $PGF_{2\alpha}$ mimic the action of NA. Inhibition of diacylglycerol lipase (by RHC 80267) and of protein kinase C (by H-7) are without effect on the potentiatory effect of NA, thus discarding a role of phospholipase C activation in this α_1-adrenergic effect. HIS, via H_1 receptors, and AD (at µM concentrations) also potentiate the increase in cAMP elicited by VIP in an indomethacin- and diclofenac-sensitive manner, hence indicating that prostanoids are also involved in this synergistic interaction. These results indicate a role for prostanoids in the amplification of the action of a peptide in the mammalian CNS.

348.2

BRAIN LEUKOTRIENE C4-BINDING SITES ARE S-ALKYL-GLUTATHIONE BINDING SITES. A. M. Goffinet. Positron Tomography Laboratory. Univ. Louvain Med. Sch. Louvain-la-Neuve, Belgium.

Recent studies (eg Goffinet & Nguyen, Eur P Pharmacol 140: 343) have demonstrated the presence of leukotriene C_4 (LTC4) binding sites in brain membranes and tissue sections and have been taken as evidence that LTC4 may serve some modulatory function in the central nervous system (CNS). So far, however, the nature and function of putative brain LTC4 receptors remain controversial.

In this work, we show that leukotriene C4 binding to mouse brain membranes is readily displaced by S-alkylglutathione derivatives, with the affinity of the test compound increasing as the alkyl chain lenght increases. S-methyl- and ethyl glutathione are equipotent with glutathione. S-butyl-, hexyl-, octyl-, nonyl- and decylglutathione show increasing activities. S-decylglutathione is almost as potent as leukotriene C4 itself.

These data strongly suggests that brain LTC4 binding sites are actually membrane-bound, glutathione-binding proteins such as glyoxalases I and II and/or, more likely, a microsomal form of glutathione transferase(s).

348.3

POSSIBLE OPIATE-SEROTONERGIC INTERACTIONS IN REPRODUCTIVE FUNCTION: LEVELS OF MU AND DELTA OPIATE RECEPTORS AFTER SEROTONERGIC LESIONS. D.L. Allen, V.N. Luine, B.S. McEwen, A.E. Johnson, A. Tempel, and R.S. Zukin. Rockefeller Univ., NY, NY; Hunter College, NY, NY; LCS/NIMH, Poolesville, MD; Albert Einstein Sch. of Med., Bronx, NY.

Opiate and serotonergic systems are important regulators of gonadotropin secretion and female sexual behavior. Interactions between these systems were examined by measuring levels of opiate receptors in the brain after selective lesions of serotonergic terminals by intraventricular infusion of 5,7-dihydroxytryptamine. Rats were sacrificed one week after the lesion. Levels of opiate receptors were measured by in vitro autoradiography using ^{3}H-[D-Ala^2,N-MePhe^4, Gly-ol^5]-Enkephalin (DAGO) and ^{3}H-[D-Pen^2,D-Pen^5]-Enkephalin (DPDPE) to respectively label mu and delta receptors . Lesions of serotonergic terminals decreased ^{3}H-DAGO binding in the POA and the MCG. Binding of ^{3}H-DPDPE to delta receptors was decreased in the POA and the DMN. Neither DAGO nor DPDPE binding was decreased in the VMN.

The decrease in binding of DAGO and DPDPE in the POA, DMN and MCG after the lesion provides evidence for interactions between opiates and serotonin in these areas. The decrease in opiate binding after the serotonergic lesion suggests that a subpopulation of opiate receptors is located on serotonergic terminals. This provides a basis for opiate regulation of serotonin turnover in the POA and MCG, and the lack of regulation in the VMN. Further studies are required to determine if the changes in opiate binding are due to changes in B_{max} or K_d. (Protocols in accordance with Federal and Society guidelines. Supported by GM07524, NS07080, and NS21973).

348.4

MONOCLONAL ANTIBODIES AGAINST CONJUGATED NEUROTRANSMITTERS AND THEIR IMMUNOCYTOCHEMICAL APPLICATIONS. J.L. CHAGNAUD, G CAMPISTRON, M.L. SOUAN, N. MONS, M.C. CHARRIER and M. GEFFARD.IBCN-CNRS, 33077, Bordeaux, France. For the simultaneous detection of chemical-defined neuronal pathway in rat central nervous system (CNS), we use poly and monoclonal antibodies directed against conjugated small-sized neurotransmitter (NT) molecules. The development of monoclonal antibodies required the following methodological aspects : (i)the mouse polyclonal immune response directed against each conjugated NT was carefully monitored using a modified ELISA method; (ii) according to the conjugated hapten, we used for the hybridization of spleen cells from immunized mice with myeloma cell lines (SP20/A0 or X63) either an adapted Siraganian's method (1983) or a modified Lane's method (1985); (iii) as soon as hybridomas producing specific antibodies against the conjugated NT could be discriminated from those producing antibodies against the coupling agent-carrier residue, affinity and specificity studies were carried out using conjugated NT closely related . We have recently raised monoclonal antibodies against : conjugated glutamate (10^{-7}M), conjugated Dopamine (10^{-9}M), conjugated Acetylcholine (10^{-10}M) and conjugated serotonin ($2x10^{-8}$M). Thanks to our poly and monoclonal antibodies and the immunocytochemical procedures, we have investigated the studies of the anatomical relationships between the various aminoacidergic, cholinergic and monoaminergic systems. The simultaneous incubation of aspartate antiserum and monoclonal anti-glutamate antibody on the glutaraldehyde-fixed sections from rat brain enables us to specifically visualize these two NT. Many combinations will further facilitate the understanding of the chemical relationships existing in the neuronal circuitry.

348.5

GABA AND ENKEPHALIN COLOCALIZE IN AMACRINE SYNAPTIC TERMINALS OF THE TURTLE RETINA. Charles L. Zucker & Alan R. Adolph. Eye Research Institute and Harvard Medical School, Boston, MA.

Enkephalin (Enk) and GABA have been localized in amacrine cells in retinas of turtle and other species. Both of these neuroactive substances have been shown to influence ganglion cell (GC) activity by functioning in concert to exert their effects. In the present study, we have used double-labeling techniques at both the LM and EM levels, as well as extracellular recording, in order to elucidate the circuitry which uses Enk and GABA to influence GC. Simultaneous LM localization of Enk and GAD showed that the majority of Enk-IR amacrine cells were, to varying degrees, also immunoreactive for GAD. Approximately 10% of the Enk-IR amacrine cell bodies were also intensely labeled for GAD. The remaining Enk-IR cells were also GAD-IR but of lower intensity. Combined pre- and post-embedding EM immunohistochemistry revealed that virtually all Enk-IR synaptic terminals were also GABA-IR. Most commonly, these GABA/Enk-IR terminals were seen to be presynaptic to GC and amacrine cells. These targets may receive additional synaptic input from GABA-IR (non-Enk-IR) amacrine cell processes. GABA/Enk-IR terminals receive input from bipolar and amacrine cell processes, some of which are GABA-IR. In the presence of functioning GABA synaptic transmission, met-Enk causes a net inhibition of GC. When GABA synapses are blocked, there is increased spontaneous and light-evoked activity in response to the opiate. Background spontaneous activity evoked by K^+ in the absence of all synaptic inputs (Co^{2+} block), is enhanced by focal met-Enk. Thus it appears that there is a direct, excitatory action of met-Enk on ganglion cells, as well as an indirect, synaptic inhibitory action that may be GABA mediated. Blockade of opiate action via naloxone, produces a broadening of ganglion cell receptive-field center and reduction of surround inhibition, based on changes in spatial transfer function measured using drifting square-wave gratings.

Supported by NIH grants EYO7552 (CLZ) and EYO3383 (ARA).

348.6

SIMULTANEOUS ULTRASTRUCTURAL DEMONSTRATION OF CHOLINE ACETYLTRANSFERASE (ChAT) AND GLUTAMIC ACID DECARBOXYLASE (GAD) IN THE RAT INPEDUNCULAR NUCLEUS. (SPON: J.G. Rutherford) M.D. KAWAJA*, B.A. FLUMERFELT AND A.W. HRYCYSHYN*. Dept. of Anatomy, The University of Western Ontario, London, Canada N6A 5C1

The ultrastructural distribution of ChAT- and GAD-immunoreactivity (IR) in the rat interpeduncular nucleus (IPN) was examined employing the sequential double antigen procedure of Levey et al. (1986). ChAT-IR was demonstrated with diaminobenzidine and GAD-IR with benzidine dihydrochloride. Each chromogen exhibited a distinct ultrastructural appearance. The central and intermediate subnuclei and the rostral half of the rostral subnucleus of the IPN possessed both ChAT-positive axons and GAD-positive somata and dendritic processes. Several types of axodendritic synapses were observed in which ChAT-positive terminals formed asymmetrical contacts on GAD-positive dendritic profiles. Symmetrical contacts between ChAT-positive terminals and GAD-positive dendrites were observed occasionally in the rostral subnucleus. Other single labeled axodendritic arrangements possessed either ChAT-positive boutons or GAD-positive dendritic processes. GAD-positive somata primarily formed contacts with non-immunoreactive terminals. This study demonstrates that the rat IPN possesses a varied array of ChAT- and GAD-positive axodendritic interactions. (Supported by the Medical Research Council of Canada)

348.7

ULTRASTRUCTURAL LOCALIZATION OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE AND GABA IN THE NUCLEUS TRACTUS SOLITARIUS. V.M. Pickel, J. Chan, C. Abate and A. Towle. Dept. of Neurology, Divs. of Neurobiology and Molecular Biology, Cornell Univ. Med. Coll., New York, NY 10021.

We examined the ultrastructural localization of the adrenaline synthesizing enzyme, phenylethanolamine N-methyltransferase (PNMT) and gamma-aminobutyric acid (GABA) in the more caudaL, cardiovascular, portions of the medial nuclei of the solitary tracts (m-NTS). Peroxidase reaction for GABA and immunoautoradiographic labeling for PNMT were principally, but not exclusively, found in different populations of perikarya, dendrites and terminals. The GABAergic neurons received synaptic input from other unlabeled terminals and from terminals containing either GABA or PNMT-like immunoreactivities. Conversely, the PNMT-labeled neurons received synaptic input from unlabeled terminals and from terminals labeled for either PNMT or GABA. In some cases, the same unlabeled dendrites received synaptic input from terminals that were immunoreactive for PNMT and GABA. These results indicate that in the m-NTS adrenergic neurons (1) modulate and are modulated by other adrenergic and GABAergic neurons and (2) share common neuronal targets with GABAergic neurons. (Supported by grants MH00078 and HL18974)

348.8

THE RELEASE OF NEUROPEPTIDES FROM SYNAPTOSOMES PREPARED FROM THE MYENTERIC PLEXUS OF THE GUINEA PIG. F.L.Christofi*, T.J. McDonald*, and M.A. Cook. Departments of Pharmacology and Toxicology, and Medicine, University of Western Ontario, London, Ontario, N6A 5C1 Canada.

A preparation of enteric synaptosomes obtained from guinea-pig myenteric plexus was used to characterize the release of Substance P-LI (SPLI), α-neurokinin-LI (α-NKLI), Galanin-LI (GALLI), Met-enkephalin-LI (MELI) and LELI. KCl failed to evoke release of any neuropeptides in the absence of selective antagonists for endogenous inhibitors. In the presence of naloxone or atropine, clear evoked release of MELI, LELI and α-NKLI was observed. In contrast, no apparent release was seen in the presence of a selective A1 adenosine antagonist. These data support an inhibitory role for ME in the release of both the opoids and α-NK and correlates with the high content of MELI measured in this preparation. Receptor-mediated release of neuropeptides was obtained with Gastrin-Releasing Polypeptide (GRP) and bombesin in the presence of naloxone. The potency orders obtained were: For α-NKLI, GRP>>Bomb; for SPLI, GRP=Bomb; for LELI, GRP>>Bomb; and for GALLI, Bomb>>GRP. These distinct profiles are consistent with heterogeneity of GRP receptors on enteric nerve endings.It is concluded that enteric synaptosomes are a suitable system for studying both release, and modulation of release by other peptides, of several neuropeptide mediators. (Supported by MRC of Canada.)

348.9

RELEASE OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY (NPY-LI) FROM THE RAT HYPOTHALAMUS. A.E. Ciarleglio*, M.C. Beinfeld* and T.C. Westfall (SPON: L.W. Harris). Dept. of Pharmacol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Neuropeptide Y (NPY) is a tyrosine rich peptide that has been previously shown by our laboratory to attenuate the release of norepinephrine (NE) from slices of the anterior and posterior hypothalamus of the rat (Westfall et al., Synapse, in press). Studies on the distribution of NPY in the central nervous system have shown that there is a dense NPY innervation of the hypothalamus particularly in the paraventricular, arcuate, and suprachiasmatic nuclei. In addition, NPY is colocalized with NE in catecholaminergic neurons of the medulla, many of which project to nuclei in the hypothalamus. The purpose of the present study was to further elucidate the role of NPY as a modulator of noradrenergic transmission in the hypothalamus by demonstrating the release of the peptide under basal and stimulated conditions. Stimulated release of the peptide from 400 μm slices of the hypothalamus of Sprague-Dawley rats was evoked by exposing the slices to 60 mM potassium for 5 min following a 50 min equilibration period and a 5 min basal period. NPY-LI was detected by radioimmunoassay using an antibody that could detect as little as 16 picograms per tube. Both basal and stimulated release of the peptide were easily detected with average stimulated release of NPY-LI 225% over basal release. (Supported by HL26319 and HL35202.)

348.10

SUBSTANCE P-INDUCED RELEASE OF EXCITATORY AMINO ACIDS FROM THE SPINAL CORD OF FREELY MOVING RATS MONITORED BY MICRODIALYSIS. D. H. Smullin, S. R. Skilling* and A. A. Larson. Dept. of Vet. Biology, University of Minnesota, St. Paul, MN 55108.

The excitatory amino acids (EAA) glutamate and aspartate and the peptide Substance P (SP) have been proposed as primary afferent neurotransmitters involved in nociceptive transmission in the mammalian spinal cord. Microdialysis of the extracellular fluid of the dorsal lumbar spinal cord has demonstrated increased concentrations of glutamate and aspartate following formalin injection (Skilling *et al.*, J. Neurochem., 1988), and that the concentration of SP increases after electrical stimulation of the sciatic nerve (Brodin *et al.*, Neurosci. Lett., 76:357-362, 1987). In the present study, the potential interaction between SP and EAA in the rat dorsal horn was investigated using microdialysis. Male, Sprague-Dawley rats implanted transversely with dialysis tubing through the dorsal spinal cord were perfused with Ringer's solution at 5 μL/min. Samples were collected in 10 min aliquots and analyzed for amino acids using HPLC with OPA derivatization. Perfusion of SP (1mM) through the dialysis tubing for 100 min produced an immediate two-fold increase in the extracellular concentration of aspartate, but no increase in glutamate, asparagine or glycine. Aspartate concentrations remained elevated throughout the perfusion period. These results demonstrate an interaction between SP and an EAA in a region of the spinal cord associated with primary afferent neurotransmission. (Supported by USPHS Grants DA04090, DA04190, DA00124, CA01342 and NIDA Training Grant DA07234)

348.11

INTERACTIONS BETWEEN TAURINE AND EXCITATORY AMINO ACIDS: RELEASE FROM CEREBRAL CORTEX SLICES FROM ADULT AND DEVELOPING MICE. Simo S. Oja and Pirjo Kontro. Department of Biomedical Scieces, University of Tampere, Finland.

Taurine is an important inhibitor of neuronal activity, in the immature brain in particular. Spontaneous and potassium-stimulated (50 mM) release of endogenous amino acids and preloaded tritiated taurine, glutamate and D-aspartate from superfused cerebral cortex slices from adult and 3-day-old mice was now monitored for 50 min. Potassium stimulation released much more taurine but less excitatory amino acids from immature than from mature cerebral cortex, the release of preloaded glutamate being about one half of that of D-aspartate in both preparations. Taurine had no effect on the release of glutamate and D-aspartate. Glutamate, aspartate and their agonists kainate, N-methyl-D-aspartate (NMDA) and quisqualate all greatly enhanced taurine release, kainate being the most effective in 3-day-old mice. Kainate also liberated much more taurine than glutamate or aspartate from both adult and developing brain. The potassium-evoked release of taurine was potentiated by kainate and NMDA, which effects were antagonized by γ-glutamyltaurine and D-2-amino-5-phosphonovalerate, respectively. The receptors for excitatory amino acids thus modify taurine release, particularly in the immature brain.

Supported by the Academy of Finland and the Emil Aaltonen Foundation.

348.12

N-methyl-D-aspartate (NMDA)-induced depolarizations and NMDA - serotonin interactions in old rat neocortex. A. Baskys, J. N. Reynolds and P. L. Carlen. Playfair Neurosci. Unit, Toronto Western Hospital, Addiction Research Foundation, Depts. of Medicine and Physiology, Univ. of Toronto, Toronto, Ont. M5T 2S8, Canada.

We examined NMDA-induced depolarizations in layer V neocortical neurons in cortical slices taken from young (4-6 mos.) and old (24-27 mos.) Fischer 344 rats. NMDA (0.5 mM) was pressure-ejected approximately 50 um dorsally from the recording site. Responses in young cells grew proportionately to the increase in the size of the NMDA droplet. In old neurons there was no correlation between the increase in the NMDA droplet size and response magnitude. Unusually large and long-lasting membrane depolarizations could be evoked in most of the old neurons by a marginal increase in NMDA droplet size. When added to the perfusate, serotonin (10 uM) significantly enhanced NMDA-induced depolarizations in young neurons but not in old. It is hypothesized that this lack of serotonin-NMDA interaction may be related to the loss of synaptic plasticity (long-term potentiation) in old rat neocortex.

Supported by Ontario Mental Health Foundation and Medical Research Council of Canada.

348.13

THE ROLE OF ACETYLCHOLINE AND NMDA MECHANISMS IN MEDIATING THE RESPONSE TO GLYCINE IN THE NTS. W.T. Talman and S.C. Robertson* (SPON: M. Hart). Lab of Neurobiology, VAMC & Univ. of Iowa, Iowa City, IA 52242.

Glycine (GLY), glutamate (GLU), and acetylcholine (ACh) microinjected into the nucleus tractus solitarius (NTS) of rat decrease arterial pressure (AP) and heart rate (HR). GLY has been demonstrated to release ACh from central tissues and to act at the NMDA GLU receptor subtype. We, therefore, sought to determine in 55 anesthetized rats if the response to microinjection (25 nl) of GLY was mediated through ACh or GLU mechanisms. Atropine (37 pmol), but not hexamethonium (1 pmol-100 n mol), blocked the response to GLY (10 nmol); eserine (16 pmol) increased the duration of the GLY response by 31%; and GLY (100 pmol) significantly increased the response to ACh (250 pmol) from a fall of AP of 11.6 ± 2.3 mmHg before GLY to 17.8 ±3.9 mmHg after GLY. In contrast, GLY (100 pmol) decreased the AP response to NMDA by 17% and in high doses (10 nmol) blocked the response to NMDA, GLU and ACh. Direct NMDA antagonism did not significantly change the response to GLY; and strychnine, which blocked the response to GLY, did not significantly affect the response to NMDA (3 pmoles). These data suggest that GLY may elicit an excitation-like response in the NTS through release of ACh and not through action on the NMDA receptor. Supported by HL32205, HL14388, NS24621, Merit Rev. Tab 18.

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION III

349.1

STRUCTURAL IDENTIFICATION, SUBCELLULAR LOCALIZATION AND SECRETION OF BOVINE ADRENOMEDULLARY NEUROMEDIN C (GRP-(18-27)). S. Lemaire, L. Chouinard*, D. Cecyre* and P. Mercier*. Dept. of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

Bombesin-like immunoreactivity (BLI) was purified from acid (HCl) extracts of bovine adrenal medulla. High performance liquid chromatography (HPLC) on a μ-Bondapak C18 column revealed the presence of five molecular forms of BLI, one coeluting with synthetic gastrin releasing peptide (GRP), the mammalian counterpart of amphibian bombesin, one coeluting with neuromedin C and one coeluting with neuromedin B, a structurally related peptide. The two other peaks of BLI eluted at times that did not correspond with those of our synthetic standards. The material corresponding to neuromedin C was purified to homogeneity and its amino acid composition and sequence corresponded to those expected for neuromedin C. HPLC analysis on an analytical SP-5PW column of subcellular extracts of bovine adrenal medulla indicated that neuromedin C is almost exclusively localized in secretory granules. The neuropeptide function of neuromedin C and/or other BLI peptides at this level was supported by the stimulatory effect of carbamylcholine (500 μM) on the release of BLI (4.5-fold increase over the basal release of 19 fmol/5 min) from perfused bovine adrenal glands. Supported by the MRCC (PG-20).

349.2

Dopaminergic Regulation of Striatal Tachykinin Gene Expression. I. Interaction of D-1 and D-2 Dopamine Receptors. M.J. Bannon, A. Rubenstein* and D.M. Haverstick. Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235.

Chronic administration of dopamine (DA) agonists and antagonists alters tachykinin (i.e. substance P and substance K) biosynthesis in the rat basal ganglia. The present experiments examined the effects of acute DA agonist treatment on striatal preprotachykinin (PPT) mRNA content. The indirect-acting DA agonist methamphetamine (METH) elicited increases in PPT mRNA which were maximal 3 hours after a single dose of 5 mg/kg. The effects of METH on PPT mRNA were prevented by concurrent administration of either the D-2 selective antagonist sulpiride (35 mg/kg) or the D-1 antagonist SCH 23390 (1 mg/kg), suggesting the involvement of both D-1 and D-2 DA receptors in this response to METH. The METH-induced increase in PPT mRNA was mimicked by the D-2 agonist quinpirole (1 mg/kg) in a SCH-reversible manner, while the D-1 agonist SKF 38393 (16 mg/kg) was without effect. These data suggest that tonic stimulation of D-1 receptors by endogenous DA is necessary for (i.e. enables) D-2 receptor activation of PPT gene expression.

349.3

Dopaminergic Regulation of Striatal Tachykinin Gene Expression. II. Mechanisms of Action. D.M. Haverstick and M.J. Bannon. Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235.

Preprotachykinin (PPT) mRNA content of rat basal ganglia is acutely increased following a single i.p. injection of methamphetamine (METH). PPT mRNA contains exons encoding for both neuropeptides substance P and substance K. Nuclease/RNA protection experiments were undertaken to determine if METH treatment induced stimulus-specific alterations in the splicing of PPT mRNA and thus any changes in the ratio of substance P to substance K since tissue-specific PPT splicing has been reported for bovine tissue. The results showed that there are two major species of PPT mRNA, they increase in a parallel manner following METH, and such an increase is blocked by pretreatment with haloperidol. By determining the amount of protectable RNA in the nuclear and cytoplasmic fractions of striatal cells it was possible to show that the METH-induced increase in PPT mRNA is first evident in the nuclei and later in the cytoplasm. These results suggest that the METH-induced increase in biosynthesis of substance P is dependent upon nuclear events such as increased gene transcription, hnRNA processing or mRNA stabilization.

349.4

THE ROLE OF ENZYMATIC PROCESSING IN THE BIOLOGICAL ACTIONS OF SUBSTANCE P. J.M. Stewart and M.E. Hall*. Dept. of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, CO 80262.

There is considerable evidence that substance P (SP) is a neurotransmitter mediating baroreceptor reflex activity in the nucleus of the solitary tract (NTS). Microinjection of SP into the NTS produces a reflex lowering of blood pressure and heart rate. The actions of synaptically released SP are thought to be terminated by enzymatic degradation, as there is no re-uptake system for intact SP. The endopeptidase 3.4.24.11 (endo 24.11) is thought to be the principle enzyme in SP degradation. The primary products of endo 24.11 activity are the amino-terminal fragment SP(1-7) (SP-N) and the carboxy-terminal fragment SP(8-11). Both are thought to be biologically inactive.

We have observed, however, that microinjection of SP-N into the NTS can reproduce the effects of injecting intact SP, suggesting to us that SP-N, rather than SP, may be the physiologically relevant transmitter molecule at this site. To test this, we examined the effects of intra-NTS injections of SP or SP-N immediately following inhibition of endo 24.11 by intra-NTS injection of phosphoramidon, a specific inhibitor of endo 24.11. We report that inhibition of endo 24.11 activity by phosphoramidon completely blocks the effects of SP, while the effects of SP-N are unaltered. It therefore appears that endo 24.11, rather than degrading SP, processes SP into a biologically active peptide, SP-N.

349.5

INFLUENCE OF THE CORTICOSTRIATAL PROJECTION ON SUBSTANCE P CELLS OF THE CORPUS STRIATUM. D.L. Somers* and R.M. Beckstead. Dept. of Anatomy and Cell Biology, Medical University of South Carolina, Charleston, SC 29425

An influence of the corticostriatal projection on striatonigral substance P (SP) cells was assessed by quantitative radioimmunocytochemistry (RIC). in rats. Cerebral cortex was removed by suction in one hemisphere from the frontal pole to the level of bregma. After 1 or 3 weeks (n=20), the brains were fixed, cut at 30 μm, and mounted onto slides. Sections were incubated in a monoclonal antibody to SP, followed by a biotinylated secondary antibody, an avidin bridge and, finally [^{3}H]biotin. The tissue was exposed to film for 14-21 days and was analyzed using an image analysis system. Optical densities were measured from whole substantia nigra (SN) on each side and converted to pmol [^{3}H]biotin/mg tissue equivalent by interpolation on a standard curve. SP levels were compared between the contra- and ipsilateral SN following cortical ablation and revealed an increase in ipsilateral nigral SP of 8.85% and 6.19% ($p<0.005$) at, respectively, 1 and 3 weeks survival. In a related experiment, striatal glutamate was depleted by more than 80% by 14 days of continuous intraventricular administration of methionine sulfoximine. Radioimmunoassay of SP revealed an increase in SN of 18% when compared to vehicle injected controls. The results suggest that corticostriatal (glutamate?) transmission plays a role in the regulation of SP levels in striatonigral neurons. Supported by NSF grant BNS 8504438.

349.6

OPIOID PEPTIDES AND SUBSTANCE P(SP), THEIR PRECURSORS AND PRECURSOR-PROCESSING ENZYMES IN HUMAN CEREBROSPINAL FLUID(CSF). D. Liu*, C. Dass*, G. Wood* and D. Desiderio. The Stout Neurosci. MS Lab, Dept. of Neurol., Univ. of Tenn., Memphis, TN 38163

Several opioid peptides, SP, their precursors, intermediate-sized precursors, and endogenous precursor-processing enzymes were found in human CSF using a combination of RP-HPLC, radioimmunoassay (RIA), and MS/MS. Methionine enkephalin(ME) and SP were identified by RIA, MS, and MS/MS in pooled human CSF following HPLC separation. Beta-endorphin(BE) immunoreactivity was measured in the appropriate HPLC fractions. Dynorphins and the C-terminal extension of ME were analyzed by hydrolyzing appropriate HPLC fractions followed by ME-RIA and LE-RIA. A fraction at 84 min in a 90-minute HPLC gradient was separated into 3 subfractions with a 120-min HPLC gradient. These subfractions were hydrolyzed with human CSF precursor-processing enzymes. ME-RIA and BE-RIA indicated the presence of precursors to ME and BE and of their processing enzymes. For unequivocal identification, the HPLC purified precursors were cleaved by CSF enzymes, the products were separated by HPLC; peptides in the HPLC fractions were determined by RIA and then identified by MS/MS. ME precursor and precursor-processing enzymes were proved strongly by MS/MS. The presence of a SP precursor and its processing enzymes was also established by RIA.

349.7

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) PRECURSOR MESSENGER-RNA IN BRAIN AND PERIPHERAL TISSUE OF RATS. J-Y. Wang, Y-L. Liu*, and A. Y-C. Shum*, Dept. of Physiology, National Defense Medical Center, and Dept. of Pharmacology, National Yang-Ming Medical College, Taipei, Taiwan, R.O.C.

Vasoactive intestinal polypeptide (VIP) is a widely distributed multifunctional neuromodulator. Northern analysis and dot blot hybridization were used to examine the biosynthesis of VIP. A Bluescribe M13+ plasmid containing an insert of 1319 bp cDNA encoding rat VIP precursor (courtesy of Dr. Tim Middleton, MIT) was transfected into E. coli, which was then transformed in M9 medium. The replicated plasmid was isolated from E. coli lysates. A 1400 bp fragment was separated by agarose gel electrophoresis from Rsa I restriction digest of the plasmid and nick-translated with [α-^{32}P] dATP to serve as the probe for hybridization with pre-pro-VIP mRNA. Cytoplasmic poly(A)+ RNA was prepared from brain, heart, intestine, and kidney of adult Sprague-Dawley rats. Northern blot autoradiograms identified a single 2000-base band mRNA. For blot hybridization, 20-ug of total RNA from each tissue was dotted onto nitrocellulose by vacuum filtration, then baked in a vaccum oven and hybridized to the same probe. The dot autoradiograms were then scanned densitometrically to quantitate mRNA. The signal in brain and intestine is at least 10-20 fold more intense than in heart and 50 fold more intense than in kidney. Experiments are underway to examine the regulation of VIP biosynthesis during the hypertensive and ischemic states. (Supported by Academia Sinica, ROC)

349.8

STEROID HORMONE REGULATION OF BRAIN NEUROPEPTIDE Y mRNA LEVELS. **P Camp and JD White. Dept Medicine, Div Endo, SUNY, Stony Brook, NY 11794.**

Estradiol (E_2) and progesterone (P_4) can induce or block LH surges in ovariectomized (OVX) rats by acting within the brain. Neuropeptide Y (NPY) can also alter serum LH levels. The goal of this study is to determine if E_2/P_4 affect NPY mRNA levels in the brain. One week after OVX (day 0), E_2 Silastic capsules were placed sc at 0900. Some rats also received P_4 capsules at 0900 on either day 0, 2 or 4. Rats were sacrificed at 1500 on day 0, 1, 2, 3 and 5. Brains were dissected and rapidly frozen on dry ice. Total RNA was isolated using an SDS/urea homogenization, repeated phenol/chloroform extraction and ethanol precipitation. NPY mRNA levels were measured using solution hybridization and nuclease digestion followed by electrophoresis in acrylamide/urea gels.

Preliminary results indicate that hypothalamic NPY mRNA levels gradually decrease with time after OVX. E_2 appears to further suppress NPY mRNA while P_4 can restore hypothalamic NPY mRNA levels depending upon the duration of treatment. Currently we are examining a more detailed time course of E_2/P_4 effects on NPY mRNA. **(Supported by NIH MH-42074 and MH-09552.)**

349.9

MODULATION OF NEUROPEPTIDE Y EXPRESSION IN CULTURED SYMPATHETIC GANGLIA R.J. Lione* and J.D. White, Div. Endocrinology, SUNY Stony Brook, Stony Brook, NY 11974

Neuropeptide Y (NPY) is contained in and released from sympathetic neurons to promote vasoconstriction. In this study, the regulation of NPY expression was investigated in co-cultures of neonatal superior cervical ganglia (SCG) and cardiac myocytes.

Dispersed cardiac myocytes are obtained from 3 day old rats by mincing heart tissue and dispase/collagenase treatment. One week later SCGs are obtained from 3 day old rats, the exterior sheath cut open and two ganglia are plated as explants/ 35mm dish. One week following SCG plating, cultures are treated or not with experimental agents. PreproNPY mRNA is measured by a nuclease protection assay. NPY peptide synthesis is assessed by incubating cultures in ^{35}S-methionine and purifying radiolabeled NPY by sequential HPLC.

These cultures were found to synthesize and release NPY. Treatment of cultures with the muscarinic agonist (+)cis-dioxolane appears to result in a 35-50% reduction in preproNPY mRNA content in ganglia whereas treatment with phorbol dibutyrate appears to increase preproNPY mRNA content by approximately 1.5-2 fold. These data suggest that preproNPY mRNA is under both stimulatory and inhibitory regulation and that such regulation can be investigated in this culture system. (Supported by a fellowship to JDW from the Aaron Diamond Foundation)

349.10

CARBOXYPEPTIDASE E (ENKEPHALIN CONVERTASE) IN THE RETINA. K.M. Braas, D.R. Lynch*, and S.H. Snyder. Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Neuropeptides are commonly synthesized as precursor molecules which are flanked by dibasic amino acids; sequential action of trypsin-like and carboxypeptidase B-like enzymes releases the biologically active peptide. Carboxypeptidase E (CPE; enkephalin convertase) (EC 3.4.17.10) cleaves single basic amino acids from the carboxyl terminal of substrate peptides. CPE is found in soluble and membrane-associated forms. Enzyme activity is cobalt-stimulated and potently inhibited by guanidinoethylmercaptosuccinic acid (GEMSA). We have examined rat retina for the presence of CPE. Soluble and membrane-associated CPE enzymatic activity and [^{3}H]-GEMSA binding were identified in retina. Enzyme activity, using [^{3}H]-benzoyl-Phe-Ala-Arg as substrate, was stimulated by cobalt and inhibited by GEMSA. The enzyme had a pH optimum of 5-6. [^{3}H]-GEMSA binding was saturable with a K_D of approximately 4 nM. Inhibitor and ion effects on enzymatic activity and [^{3}H]-GEMSA binding were similar to those reported with purified bovine pituitary soluble and membrane-associated CPE. CPE messenger RNA was demonstrated by Northern analysis using cDNA probes to bovine pituitary CPE. Autoradiography using [^{3}H]-GEMSA and immunocytochemistry using anti-CPE localized highest levels in the inner plexiform layer of the retina.

349.11

DISTRIBUTION OF PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE (PAM) IN NEURONAL AND PERIPHERAL TISSUES. V. May, K. M. Braas and B. A. Eipper. Neuroscience Dept., Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Peptidylglycine α-amidating monooxygenase (PAM; EC 1. 14.17.3) catalyzes the formation of bioactive α-amidated peptides from their glycine-extended precursors. A bovine PAM cDNA encodes a 108 kDal protein with an N-terminal signal sequence, followed by a propeptide and the catalytic domain, with a putative transmembrane domain near the C-terminus. Post-translational processing of the precursor at dibasic amino acids may yield a soluble form of PAM. The levels and forms of PAM mRNA in rat tissues were determined by Northern analysis using bovine PAM cDNA probes. In the CNS, the level of PAM mRNA was highest in the hypothalamus with moderate levels in cerebral cortex, striatum, hippocampus, brainstem, thalamus and retina; low levels were found in olfactory bulb and cerebellum. Heart atrium contained the highest level of PAM mRNA of all tissues; high levels were also identified in anterior and neurointermediate lobes of the pituitary gland, and in heart ventricle. Moderately high levels of PAM mRNA were observed in the submaxillary, sublingual and thyroid glands, while low levels were found in the ovary, adrenal gland, lung and kidney. The ratio of soluble to membrane-associated PAM enzymatic activity was tissue specific.

349.12

IN VIVO SOMATOSTATIN BIOSYNTHESIS IN THE RAT CEREBRAL CORTEX. R.P.S. Kwok and J.D. Fernstrom. Departments of Psychiatry and Behavioral Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh PA 15213.

The cerebral cortex contains a major fraction of the total somatostatin (SRIF) immunoreactivity (IR) in the central nervous system. In this region, IR-SRIF is located in neurons, suggesting a neurotransmitter function. SRIF biosynthesis can be studied in vivo in rat hypothalamus following ^{35}S-cysteine administration into the third ventricle (Van Itallie & Fernstrom, ENDOCRINOLOGY 113: 1210, 1983). We have now adapted this technique to study SRIF biosynthesis in rat cerebral cortex. Anesthetized male rats (250-300 g) received 100 microCi ^{35}S-cysteine into each lateral ventricle, and were killed 5 hr later. A bilateral strip of cortex around the injection sites was removed from each rat, and prepared for HPLC separation. The samples were subjected to two sequential reversed-phase HPLC separations, to isolate labeled SRIF peptides (SRIF-14 and SRIF-28). Following this procedure, a prominent ^{35}S-cysteine peak was found corresponding to SRIF-14. Only a very small SRIF-28 peak was observed. The SRIF-14 peak was further verified by showing it to shift its retention time following reduction and carboxymethylation to that corresponding to standard carboxymethyl-SRIF-14. Labeling of SRIF-14 was also observed to be suppressed in rats pretreated with cysteamine, as it is in hypothalamus. These and other data suggest it is now possible to study in vivo the kinetics of SRIF biosynthesis in the cerebral cortex. These methods should prove useful in elucidating the role of SRIF peptides in cerebral function.

349.13

The primary neuron-enriched culture from rat brain as a model for studying somatostatin (SS) and neuropeptide Y (NPY) turnover. *J.J. Poulakos, W.J. Millard, M.K. Raizada and E.M. Meyer, Univ. of Florida, Sch. of Med., Dept.of Pharmacol. & Therapeutics, Gainesville, Fl. 32610*

We recently found that long term cholinergic hypofunction induced by bilateral lesioning of nucleus basalis induces greatly elevated NPY, SS and CRH levels in the cerebral cortex, which appear to occur concomitantly with memory-related behavioral recovery; suggesting a potential role for these peptides in memory. Since these elevations could be due to increased synthesis or reduced release, we began to investigate SS release (measured by RIA) and NPY synthesis (Northern blot analysis, probe provided by Dr. J.Allen) in neuronal cell cultures. SS was found to be synthesized (5.3±0.3ng/mg protein) and then released in a depolarization-sensitive manner. Norepinephrine (100μM) and insulin (0.1μg/ml) triggered release, while carbachol (50μM) had no effect. Atropine (1μM) however, increased release, consistent with the presence of cholinergic synapses on SS neurons. With respect to peptide synthesis, Northern blot analysis revealed high levels of NPY-mRNA in 19 day old cultures, indicating that it should be possible to characterize further NPY expression. These studies will be important in developing a unifying hypothesis to account for how changes in one ascending pathway affect three different neuropeptide-systems similarly.

349.14

ISOLATION OF A 7 KILODALTON PEPTIDE DERIVED FROM BRAIN PROSOMATOSTATIN. R.A. Benoit and G. Gravel*. Department of Medicine, Montreal General Hosp. Research Institute and McGill University, Montreal, Que. H3G 1A4.

The largest peptide product derived from mammalian prosomatostatin (proSS) is an 8 kilodalton (kD) molecule that we identified in rat brain as preproSS(25-100). We have indicated that certain tissue extracts contain an abundant molecular form related to the 8 kD peptide and now report its isolation from brain. The acid extract of 210 rat brains containing peptidase inhibitors was fractionated on Sephadex G-75 gel permeation chromatography columns and the effluent was monitored by radioimmunoassay (RIA) for the presence of peptides containing the first eight amino acids at the NH_2-terminus of prosomatostatin. A somatostatin-28(1-12) RIA was used to identify preproSS-(25-100). The two main immunoreactive peaks eluted from the column were a 7 kD material and preproSS(25-100). These two molecular forms could only be resolved by ion-exchange chromatography on carboxymethyl cellulose (CMC) at low ionic concentration. The 7 kD material from CMC was further purified by reversed-phase liquid chromatography (RPLC) using heptafluorobutyric or trifluoroacetic acid /acetonitrile as the mobile phases. Three immunoreactive components were seen by RPLC. The major one was rich in Ala and Leu and contained a single met. These results indicate that the 7 kD peptide(s) represents a major product of prosomatostatin processing in brain and that it is heterogeneous. Supported by canadian MRC and FRSQ.

349.15

A CRITICAL ANALYSIS OF THE USE OF CYSTEAMINE TO DEPLETE SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM (SRIF-LI). L.L. Cook*, G. Bissette, and C.B. Nemeroff (SPON: D.L. Evans). Depts. of Psychiat. & Pharmacol., Duke Univ. Med. Center, Durham, NC 27710.

The wide CNS distribution of SRIF-LI and its reduction in Alzheimer's disease have stimulated studies of its extrahypothalamic role. The reduction of hypothalamic SRIF-LI by subcutaneously (SC) administered cysteamine (CYS) is well-documented, but reports describing effects of centrally administered CYS on SRIF-LI are discordant. SRIF-LI was measured by RIA in the frontal cortex (CTX), hypothalamus (HYP), and hippocampus (HIP) in rats following daily (x7) infusions into stereotaxically positioned, unilateral cannulae in either the lateral ventricle (LV, 300 $\mu g/2\mu l$) or the dorsal HIP (100 $\mu g/2\mu l$), and following single (300 mg/kg) or daily (100 mg/kg, x7) SC injections; rats were killed 4 or 24 hr after the last injection. Following LV infusions, SRIF-LI was reduced only in the HYP (35% at 4 hr and 27% at 24 hr; $p<0.05$). Following HIP infusions, SRIF-LI was reduced only in the HYP at 4 hr (23%) and was not changed in either the ipsi- or contra-lateral HIP or CTX. Although the depletion of SRIF-LI in the HYP was increased by repeated SC dosing, the extrahypothalamic reduction of SRIF-LI by SC CYS was negligible and was not enhanced by repeated dosing. CYS does not appear to be a particularly effective agent for the reduction of extrahypothalamic SRIF-LI following SC or CNS administration. (Supported by NIMH MH-40524 and NIA AG-05128)

MESSENGER RNA REGULATION III

350.1

ANTI-DEPRESSANT DRUGS INCREASE GLUCOCORTICOID RECEPTOR mRNA IN PRIMARY CULTURES OF RAT BRAIN NEURONS. Marie-Claude Pépin* and Nicholas Barden, Ontogénèse et Génétique Moléculaires, Le Centre Hospitalier de l'Université Laval, Ste-Foy, Québec G1V 4G2, Canada.

Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, indicated by elevated serum cortisol levels and a non-suppression of serum cortisol following dexamethasone (DEX) administration are frequently associated with major depressive illness. The hyper-cortisolaemia of depression is not associated with any of the physical characteristics of Cushing's syndrome and this apparent lack of sensitivity to glucocorticoids may be due to a reduction in glucocorticoid receptor (GR) number. A decreased number of GR in lymphocytes of depressed patients has been noted and, if such a decrease is also present in the CNS, this could explain the lack of feedback regulation characteristic of non-suppressors. Since non-suppressive responses of serum cortisol to DEX revert to normal during anti-depressant therapy, it is possible that anti-depressant drugs modify the GR content of brain areas involved in control of the HPA axis. To test this hypothesis, we investigated the effects of anti-depressants on GR mRNA in cultured rat brain neurons.

Hypothalamus, cerebral cortex and amygdala of 16-18 day rat embryos were excised under a dissecting microscope. Dissociated cells were cultured in serum- and antibiotic-free, chemically defined, medium (DMEM-F12). After 15 days in culture, cells were incubated with the test substance for 48 hours and their RNA content isolated and deposited on nylon filters. The filters were hybridized with a ^{32}P-labelled RNA probe complementary to a 2,2 Kb fragment of the GR cDNA. Actin mRNA concentration was used as internal standard.

Irrespective of selective inhibitory actions on reuptake of serotonin (amitriptyline, imipramine, trimipramine) or norepiniphrine (maprotiline, desipramine), all anti-depressants tested increased the GRmRNA level of hypothalamic cells. In cultures derived from cerebral cortex or amygdala, the level of GRmRNA was increased by amytriptyline and desipramine, while imipramine was without effect. These results indicate that anti-depressants can modulate the GR receptor content of brain areas involved in control of the HPA axis, and suggest a mechanism of action for their normalization of endocrinological parameters in depressive patients.

350.2

GLUCOCORTICOID AND MINERALOCORTICOID RECEPTOR mRNA EXPRESSION IN RAT BRAIN. H.M. Chao* and B.S. McEwen. (SPON: A. Miller) Lab. of Neuroendocrinology, Rockefeller Univ., N.Y.,N.Y.10021

The autoregulation of corticosterone receptors by glucocorticoids is well documented. The binding of CORT in the rat brain is mediated by two receptor types, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Molecular probes for the GR and MR were used to study the regulation of these receptors at the mRNA level. GR mRNA expression was quantified using a rat GR probe (from K. Yamamoto) in a SP6 quantitative nuclease protection assay which employs RNAse T2. The levels of expression of GR mRNA are comparable in the cerebellum, hippocampus, hypothalamus, pons-medulla and striatum. Adrenalectomy (ADX) induces an increase and subsequent CORT replacement causes a decrease in cytosolic GR binding; however, no significant changes in the GR mRNA levels are seen with these treatments. We conducted similar analyses using a cDNA clone for the MR (from R. Evans). Though the MR is barely detectable in intact rats in the cytosolic receptor binding assay, the MR mRNA is readily measured and shows a higher level of expression in the hippocampus than in the other four brain regions examined. Initial indications are that the steady-state level of expression of the MR mRNA (like that of the GR mRNA) is not altered by ADX or CORT treatment. (Supported by MH41256 and NS07080.)

350.3

TWO TYPES OF GLYCINE RECEPTORS EXPRESSED IN XENOPUS OOCYTES INJECTED WITH RAT SPINAL CORD mRNA. H. Akagi* and R. Miledi, Laboratory of Cellular and Molecular Neurobiology, Department of Psychobiology, University of California, Irvine, CA 92717.

Poly(A)$^+$-messenger RNAs (mRNAs) isolated from adult and neonate (3-4d old) rat spinal cord were injected into Xenopus oocytes which were subsequently examined under voltage clamp. Messenger RNAs obtained from both sources induced the oocytes to aquire functional glycine receptors (GlyR). The glycine-induced currents reversed direction at about -20mV (close to the equilibrium potential of Cl^- in the oocytes) and the responses were blocked by strychinine (0.5μM) or picrotoxin (20μM). Thus, the GlyR encoded by two kinds of mRNAs share some common characteristics. Nevertheless, size fractionation of the GlyR-mRNAs by sucrose density sedimentation exhibited contrasting profiles; the majority of the adult cord GlyR-mRNA sedimented in heavy density fractions, close to the position of the 28S RNA, whilst the neonate GlyR-mRNA was seen mainly close to that of the 18s RNA. The properties of GlyR encoded by the adult mRNA were different from those of the receptor encoded by the neonatal mRNA in respect to dose-response relationship, time-course of desensitization and some pharmacological actions. These results suggest that in rat spinal cord there exist, at least, two molecular classes of mRNAs which code for distinct glycine receptors. Moreover, the production of the GlyR-mRNAs in the spinal cord appears to be developmentally regulated.

350.4

REGULATION OF TYROSINE HYDROXYLASE (TH), PHENYLETHANOLAMINE N-METHYLTRANSFERASE (PNMT) AND PROENKEPHALIN (pEK) mRNAs IN ADRENAL MEDULLARY CELLS BY CATECHOLAMINE DEPLETING AGENTS. ROLE OF TRANSCRIPTION AND PROTEIN SYNTHESIS. O.H. Viveros, J.S. Hong, J. Sigafoos, and M.K. Stachowiak. Borroughs Wellcome Co. and NIEHS/LMIN, RTP, NC 27709.

Depletion of catecholamines (CA) in cultured bovine adrenal medullary cells (BAMC) increases the content of TH and pEK derived peptides and modifies TH, pEK and PNMT mRNAs levels. Incubation of BAMC with tetrabenazine (TBZ), 0.1-100 μM, for 48 hr produced a dose-dependent depletion of CA and a inversely proportional increase in TH and pEK mRNA levels. Reserpine produced similar results suggesting that the levels and/or intracellular distribution of the CA regulate the expression of TH and pEK genes. The level of PNMT mRNA exhibited complex responses to TBZ and reserpine. Increases (+50 to +100%) were observed at low concentrations of the drugs but decreases (-40 to -94%) at the higher concentrations. TBZ at 100μM produced biphasic time-dependent changes in pEK mRNA; a 2-3 fold increase at 3 hours was followed by a gradual decline (-90% at 48 hours). The increase in TH mRNA (+420%) by TBZ was not affected by cycloheximide (+408%) but reduced to +82% in the presence of α-amanitin (both drugs at 10μg/ml), suggesting transcriptional regulation of TH. In contrast, both the increase in pEK and the biphasic responses of PNMT mRNA after CA-depleting drugs, were prevented by cycloheximide, indicating involvement of protein synthesis in the regulation of these two adrenomedullary proteins.

350.5

PROLONGED TREATMENT OF PC12 CELLS WITH POTASSIUM REDUCES TYROSINE HYDROXYLASE mRNA LEVELS. E.J. Kilbourne* and E.L. Sabban. Dept. of Biochem., New York Med. Coll., Valhalla, NY 10595.

To study the effect of prolonged neuronal activity, we examined the effect of long-term treatment of PC12 cells with potassium. Cells were treated for 3 days with 56 mM KCl. Morphologically, the general cellular appearance in phase contrast miroscopy was unaltered. The cellular dopamine and norepinephrine were completely depleted, as expected. Total RNA was extracted from the cells and analyzed by Northern blot analysis, and hybridized with a ^{32}P labelled cDNA to tyrosine hydroxylase (TH). The TH mRNA levels were decreased over 10 fold.

The time course of this effect showed that the TH mRNA levels was not decreased upon treatment for 1 day or less with 56 mM KCl, but from 2-5 days it went down over 10 fold. The specificity of effect was examined by assesing actin mRNA levels and total protein synthesis under these conditions. Hybridization with actin cDNA showed no decrease in actin mRNA following this treatment. Moreover, protein synthesis, measured by incorporation of ^{35}S-methionine into protein, was similar at these time points. Thus, there appears to be a large specific decrease in TH mRNA levels upon prolonged treatment of PC12 cells with 56 mM KCl. (Supported by NIH grant NS 20440)

350.6

CO-REGULATION OF TYROSINE HYDROXYLASE AND INSULIN mRNA IN PRIMARY CULTURES OF RAT PANCREATIC ISLET CELLS.
D.R. Studelska*, C.A. Marshall*, C.J. Fink*, M.L. McDaniel*, and K.L. O'Malley (SPON: R. Weinshilboum).
Anatomy/Neurobiology and Pathology Depts., Washington University School of Medicine, St. Louis, MO 63110

It has been shown that tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine production, is expressed in a subset of insulin-secreting pancreatic islet cells (Teitelman and Lee, Dev. Biol., 121: 454, 1987). We have recently shown that the human TH gene is within 2.7 kb of the insulin (INS) gene on chromosome 11. Such close linkage raises the possibility that factors which influence one gene might act upon the contiguous gene. As the first step in testing this hypothesis, we have isolated rat pancreatic islet cells, maintained them in dissociated culture and tested for TH or INS mRNA expression using in situ hybridization in conjunction with immunological techniques. Preliminary experiments suggest that both INS and TH mRNA expression are dependent on glucose concentration. Six hr after plating in media containing 16.5 mM glucose, INS-containing islet cells expressed high levels of INS mRNA. In parallel cultures, TH mRNA was detected in a subset (30%) of INS immunoreactive cells. The levels of TH mRNA in these cells ranged from 5-30% of the average amount of INS mRNA expressed under comparable conditions. Under low glucose (5.5 mM) conditions, both INS and TH message could be detected at lower levels in islet cells cultured for 1-6 hours, but after three days, fewer than 0.1% of cells expressed INS mRNA, while TH message could not be detected. In contrast, islet cells maintained in 16.5 mM glucose produced both messages after all time intervals. These data suggest that TH and INS are coordinately regulated by glucose. We are currently determining if other factors known to modulate TH gene expression also co-regulate INS levels.

350.7

IN SITU HYBRIDIZATION ANALYSIS OF THE LOCALIZATION AND REGULATION OF DARPP-32 IN THE RAT BRAIN . M. Ehrlich, M. Schalling, M. Djurfeldt*, M. Herrera-Marschitz*, H. Hallman*, T. Kurihara*, T. Hokfelt, and P. Greengard. The Karolinska Institutet, Stockholm, SW and The Rockefeller University, NY, NY.

Dopamine- and adenosine-3':5'monophosphate-regulated phosphoprotein (DARPP-32) is highly enriched in neurons bearing D1-receptors, particularly the medium spiny neurons of the caudate nucleus. We investigated the effects of dopamine-related agents on steady-state levels of DARPP-32 mRNA in rats via in situ hybridization with an ^{35}S-labelled oligonucleotide probe complementary to rat DARPP-32 mRNA. In control animals, the distribution of DARPP-32 mRNA correlated with that of the protein as previously shown by immunocytochemistry. After overnight autoradiography, a strong signal was observed in the caudate nucleus, nucleus accumbens, pyriform cortex and ependymal cells surrounding the third ventricle. The distribution in the caudate was somewhat patch-like. Longer exposure revealed labelling in two bands throughout the cerebral cortex. The various treatments had little or no effect on the level of DARPP-32 mRNA, but the specific mRNAs for substance P, dynorphin, or glutamic acid decarboxylase were markedly altered from control. We conclude that the steady-state level of DARPP-32 mRNA is more stable and is regulated by different mechanisms than are the levels of mRNA for several other molecules involved in signal transduction in the caudate nucleus.

350.8

PROTEIN SYNTHESIS INHIBITION BLOCKS THE INCREASE IN LEVELS OF TYROSINE HYDROXYLASE-mRNA INDUCED BY cAMP OR GLUCOCORTICOID IN A RAT PHEOCHROMOCYTOMA CELL LINE. L.H. Fossom*, N. Weiner and A.W. Tank, Depts. Pharmacology, Univ. Colorado HSC, Denver, CO 80262 and Univ. Rochester Med. Ctr., Rochester, NY 14642. (Sponsor: Victor G. Laties)

We have previously reported that both cAMP and glucocorticoid induce levels of tyrosine hydroxylase (TH) enzyme in PC18 cells, and that this induction is preceded by and can be accounted for quantitatively by increased synthesis rate of TH and increased levels of TH-mRNA. We and others have shown that the induction of TH-mRNA levels is due in part, if not entirely, to increased transcription of the gene for TH. In this report we show that two protein synthesis inhibitors, that work by different mechanisms, can block the induction of TH-mRNA by either cAMP or dexamethasone. Three hour treatments with either 1 mM 8Br-cAMP or 1 μM dexamethasone increased TH-mRNA levels 2.5- or 2.9-fold, respectively. Treatment with 3.7 μM cycloheximide beginning 1 hr before and continuing during induction, completely blocked these increases in TH-mRNA without altering basal levels. Protein synthesis inhibition using 37 μM puromycin also blocked induction of TH-mRNA by both inducers. These results suggest that inhibition of the synthesis of some rapidly turning over protein(s) prevents induction of TH-mRNA by cAMP and dexamethasone. We are currently working to determine whether this protein factor(s) is required for increased transcription of the TH gene or acts to stabilize TH-mRNA.

350.9

DOPAMINE DECREASES TYROSINE HYDROXYLASE mRNA IN CULTURED RAT ADRENAL MEDULLA. W.J. Burke, R. Strong, H.D. Chung, A.C. Towle, H.P. Paivarinia, G.L. Marshall and T.H. Joh. St. Louis VAMC, St. Louis Univ. Med. Sch., St. Louis, MO 63125 and Cornell Univ. Med. Coll., New York, NY 10021.

We have recently reported that dopamine (DA) reduces the activity amount and rate of synthesis of tyrosine hydroxylase in cultured rat adrenal medulla (Biochem. Pharmac. 37, 1391-1398, 1988).

In the present study we sought to determine if the decrease in TH synthesis was due to decreased levels of mRNA. Adrenal medullae from 4 month old 450g Holtzman rats were cultured in the presence or absence of 2mM (DA) for 22h. TH mRNA was determined by in situ hybridization using a [^{32}P] labelled oligonucleotide probe. Densitometric quantification of the X ray film revealed a 56% decrease in TH mRNA in DA treated medullae. In a similar experiment 2mM epinephrine resulted in a 30% decrease in TH mRNA. Northern blot analysis of medullae exposed to DA also revealed a 70% decrease in TH mRNA. Thus DA reduces TH synthesis by reducing TH mRNA in cultured adrenal medullae.

350.10

TRANSLATIONAL REGULATION OF TYROSINE HYDROXYLASE IN RAT PHEOCHROMOCYTOMA CELLS. S.A. Signs*, J.F. Bowyer and A.W. Tank, Depts. Pharmacology, Univ. Rochester Med. Ctr., Rochester, NY 14642 and Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

PC18 pheochromocytoma cells display 3-4 fold increase in tyrosine hydroxylase (TH) and TH-mRNA after treatment with dexamethasone (DEX) or 8-bromo-cyclic-AMP (8Br-cAMP). When PC18 cells are treated simultaneously with DEX plus 8Br-cAMP, additive amounts of TH enzyme (i.e. 6-8 fold) are synthesized, however, TH-mRNA levels are not increased additively. The synthesis of ^{3}H-TH in cell-free reticulocyte lysates directed by RNA isolated from DEX plus 8Br-cAMP treated cells is 2-fold greater than that directed by RNA containing equal amounts of hybridizable TH-mRNA from cells treated with either inducer alone (Bowyer et al., submitted for publication). In an effort to explain this increased translational activity of TH-mRNA, we have used a primer extension assay to examine whether multiple TH-mRNA transcripts are produced. ^{32}P-Labeled oligomer complementary to 20 nucleotides near the 5'-end of exon 2 of TH-mRNA was annealed to RNA isolated from each treatment condition and the RNA·DNA hybrids were extended using AMV reverse transcriptase. Oligomer-TH-mRNA hybrids from all treatments extended to the same predicted transcriptional start-site. Thus, enhanced translational activity of TH-mRNA derived from DEX plus 8Br-cAMP treated cells is not due to an alternate transcriptional start-site or to alternate splicing to form multiple first exons.

350.11

GROWTH HORMONE GENE EXPRESSION IN GENETICALLY OBESE MALE AND FEMALE RATS. I. Ahmad*, A.W. Steggles*, and J.A. Finkelstein. Depts. of Anatomy and Biochemistry, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

Pulsatile release of growth hormone (GH) is significantly decreased in male genetically obese Zucker rats (fa/fa) in comparison to lean littermates (Fa/-). In order to understand the underlying molecular mechanism of this abnormality, we have begun studying GH gene expression. Pituitaries were obtained from seven pairs of obese and lean littermates. After homogenization of the pituitaries, GH mRNA was quantified by cytoplasmic dot blot, using a 32P-labeled cDNA probe. In the male animals, GH mRNA level was 2.5 fold lower in the obese rats than in the lean controls. A similar comparison between groups of female rats of the same age showed a change in the same direction but the difference was not as pronounced as that seen in the groups of male animals. The variability within the groups of females was greater than that seen in the groups of male animals. Although obesity is as marked in the females as in the males, sex hormones may influence the observed differences in GH mRNA levels between obese and lean animals. (Supported by Ohio Board of Regents Research Challenge Funds).

350.12

DEVELOPMENTAL CHANGES IN RAT ANTERIOR AND INTERMEDIATE POMC PRIMARY RNA TRANSCRIPT AND MATURE MESSENGER RNA. R. Scott*, D.J. Autelitano, M. Blum, J.L. Roberts, and J.E. Pintar (SPON: M. Ferin). Dept. Anatomy and Cell Biology, Columbia P&S, NYC, NY 10032 and Fishberg Ctr. for Neurobiology, Mt. Sinai Medical Center, NYC, NY 10029.

Anterior and intermediate lobe proopiomelanocortin (POMC) cells are derived from a common embryological precursor, but differentiate at different times during development and are characterized by developmental changes in the extent of prohormone processing. In this study we have determined the levels of POMC primary transcript and POMC mRNA in separated anterior and neurointermediate (NIL) lobes at late prenatal and early post-natal stages of rat development using POMC intron/exon splice junction probes. Anterior and NIL lobes were dissected from fetal (e18,e21), neonatal (p1,p10,p21), and adult Sprague-Dawley rats and nuclear and cytoplasmic fractions isolated. Radiolabelled antisense cRNA probes were synthesized from an SP65 vector containing a rat genomic DNA fragment encoding all of POMC exon 1 and 60 bp of intron A. Radiolabelled probe was hybridized to ioslated nuclear and cytoplasmic RNA in solution, digested with RNAse or S1 nuclease following hybridization, and separated by gel electrophoresis. POMC RNA species were quantitated by comparison to a standard curve produced by hybridizing radiolabelled antisense RNA to unlabelled sense RNA. Although the amount of POMC primary transcript per pituitary increased in both cell populations during these ages, (e.g. 0.03 fmole/anterior pituitary (AP) at e18 to 0.17 fmole/AP in the adult), POMC mRNA levels increased by greater amounts in both lobes. Thus, the relative abundance of POMC primary transcript compared to mature POMC mRNA in both anterior and NIL was markedly higher during late gestation than in the adult, which indicates that dynamic changes in POMC transcript processing are occurring during differentiation of both POMC cell populations during development. Supported by NIH grants to JP and JLR.

350.13

EFFECT OF CHEMICAL ADRENALECTOMY ON VASOPRESSIN, CRF, AND POMC mRNA REGULATION IN THE HYPOTHALAMUS AND PITUITARY. S.P. Kwak*, E.A. Young, R. Przewlocki*, H. Akil, and S.J. Watson (SPON: M.J. Majchrzak). Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Adrenalectomy has often been used as a paradigm for studying the role of glucocorticoid negative feedback on the hypothalamo-pituitary system. However, using this procedure it is difficult to study acute effects, since confounding variables such as surgical stress and steroid release during removal of adrenals are often associated with this procedure. To observe the acute consequences of endogenous glucocorticoid removal, we have employed metyrapone as a potent inhibitor of adrenal steroid synthesis and studied the changes in mRNA levels in relevant nuclei. Rats were treated with daily injections of metyrapone (20mg/100g bw) s.c. for 3 days, sacrificed, and the brain was microdissected using a brain block. Using RNase protection assay, we observed 200% increase of POMC mRNA over controls in the anterior pituitary, confirming our previous data obtained from dot blots. Vasopressin mRNA increased by 62% in the PVN. Plasma β- endorphin levels were also elevated 3 fold over controls, suggesting that the effects produced by metyrapone resembles changes observed during adrenalectomy. Our data is in agreement with the study by Plotsky et al. (*Endocrinology*, 120(4)1987), showing 5.2 fold rise in plasma ACTH levels with concurrent increase in CRF and vasopressin staining in the PVN. We are currently assaying for CRF mRNA and plasma levels of β-endorphin over various days of treatment to compare the effects of chemical versus surgical adrenalectomy.

350.14

OXYTOCIN GENE EXPRESSION IN THE SUPRAOPTIC NUCLEUS DURING PREGNANCY AND LACTATION P.J. Brooks, P.K. Lund*, W.E. Stumpf, and C.A. Pedersen. The Curriculum in Neurobiology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC, 27514

We have investigated the regulation of oxytocin gene expression in the supraoptic nucleus on days 10,18, and 22 of pregnancy and day 5 of lactation in the rat using quantitative in situ hybridization with an I125 tailed oligonucleotide probe. For each treatment group, frequency distributions relating grain densities to percent of total labelled cells were generated by an observer unaware of treatment conditions. Examination of the frequency distribution of the lactating group compared to the pregnant groups indicated a shift to the right, i.e. an increased percentage of cells having higher grain densities in the lactating group compared to all of the pregnancy groups. The shape of the distribution of the lactating group was found to be significantly different from all other groups (Kolmogorov-Smirnov Test, $p<0.001$).

These results indicate that oxytocin mRNA levels in supraoptic nucleus cells remain relatively constant over late pregnancy, while lactation is associated with an increased percentage of cells in the supraoptic nucleus expressing higher mRNA levels. We are presently determining whether oxytocin cells in other brain regions undergo similiar changes.

350.15

REGULATION OF PROENKEPHALIN (pEK) mRNA IN RAT ADRENAL MEDULLA (AM) DURING STRESS AND AFTER ADRENAL DENERVATION. IN SITU HYBRIDIZATION STUDY. M. Sar[a]*, J.S. Hong[c], W. Stumpf[a], B.B. Kaplan[b], L. Thai[c]*, E.K. Stachowiak[a]*, and M.K. Stachowiak (SPON: G.J. Harry). University of North Carolina, Chapel Hill, NC[a], University of Pittsburgh, PA [b], NIEHS Res. Triangle Pk., NC[c] 27709.

We have examined the regulation of pEK gene expression in AM cells using a single-stranded DNA probe complementary to 30 nucleotides of the coding region of pEK gene. Cells expressing pEK mRNA were unevenly distributed in AM, and were not found in the adrenal cortex. Transection of the splanchnic nerve increased 4-8 times pEK mRNA content in the individual AM cells. Increases occured predominantly in the portion of medulla adjacent to the nerve transection. Intensity of hybridization signals was also affected by hypoglycemia. 24 hrs following 2 hrs insulin shock pEK mRNA levels in individual AM cells increased 5-10 fold. These changes were distributed throughout the medulla. No changes in the number of cells expressing pEK mRNA were detected. Taken together our results indicate that the neural regulation of pEK gene expression in AM cells is complex. Under basal condition neural input appears to have an inhibitory effect, whereas elevation of the nerve activity by hypoglycemia enhances pEK gene expression.

350.16

HALOPERIDOL AND DOMPERIDONE TREATMENTS HAVE THE OPPOSITE EFFECT ON LEVELS OF PROENKEPHALIN mRNA IN THE CAUDATE-PUTAMEN OF THE RAT BRAIN. C.S. Woolley*, J.A. Angulo and B.S. McEwen (SPON: G. Ryan). Lab of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

In situ hybridization histochemistry was used to evaluate the effects of two pharmacological agents with D2 dopamine receptor subtype antagonistic activity, haloperidol and domperidone, on levels of proenkephalin (PE) mRNA in the mid caudate-putamen (C-PU) region of the rat brain. Male Sprague-Dawley rats were treated for six days with haloperidol (1 mg/kg/day) or domperidone (0.4 and 0.8 mg/kg/day) delivered by osmotic minipump. A synthetic oligonucleotide (30mer) complementary to regions in the first and second exons of the PE message was end-labeled with ^{32}P and used as a hybridization probe to detect specific mRNA in coronal brain sections from treated and control rats. Optical density of X-ray film exposed by labeled sections was quantitated in the C-PU region by densitometry using an image analyzer and data were expressed as percent of control values. Treatment with haloperidol resulted in a 2.5 fold increase in the level of PE mRNA while treatment with domperidone resulted in decreased levels. Thus, the effect of haloperidol treatment on PE mRNA in the C-PU region of the rat brain is not equivalent to the antagonistic effect of domperidone at the D2 dopamine receptor subtype.

350.17

EFFECT OF SODIUM DEPLETION AND ALDOSTERONE TREATMENT ON ANGIOTENSINOGEN mRNA IN THE BRAIN OF THE RAT. J. Angulo, J. Schulkin* and B. McEwen, Dept. of Anatomy, University of Pennsylvania and Lab. of Neuroendocrinology, The Rockefeller University.

We have studied the effect of sodium depletion and aldosterone treatment on brain angiotensinogen mRNA by in situ hybridization histochemistry using a synthetic oligonucleotide probe corresponding to amino acids 106-115 of proangiotensinogen. The probe was radiolabelled at the 3' end by the addition of adenosine mononucleotides (32-P) by terminal deoxynucleotidyl transferase.

Sodium depletion resulted in increased levels of angiotensinogen mRNA in the preoptic area and no changes in the dorsomedial and ventromedial nuclei of the hypothalamus or the medial amygdala. Aldosterone treatment increased angiotensinogen mRNA levels in the preoptic area but not in other brain areas examined. The data suggest that sites in the preoptic area of the brain are part of a neural circuit involved in regulating the hunger for salt.

350.18

BRAIN ANGIOTENSINOGEN mRNA IN GENETICALLY HYPERTENSIVE AND NORMOTENSIVE RATS. B.G. Yongue, J. Angulo and B.S. McEwen. New York State Psychiatric Insitute and Rockefeller University, New York, NY 10021

Brain angiotensin is implicated in blood pressure (BP) control and fluid/electrolyte metabolism, via vasopressor actions and stimulation of thirst and salt consumption. Spontaneously Hypertensive rats (SHR) not only have high BP, but also consume more salt than Wistar/Kyoto rats (WKY), a normotensive strain from the same progenitor. SHR also seem to be more responsive than WKY to both the BP and salt appetite stimulating actions of the renin-angiotensin system. We are examining genotype and adrenal influences on expression of the gene coding for angiotensinogen, the angiotensin precursor, in brain of SHR and WKY. Brains of SHR and WKY, either intact or adrenalectomized, were frozen and sectioned at 16 u. Brain angiotensinogen mRNA was detected by in situ hybridization histochemistry with a synthetic oligonucleotide probe (proangiotensinogen, amino acids 106-115). The probe was radiolabelled by addition of adenosine mononucleotides (32P) at the 3' end by terminal deoxynucleotidyl transferase. Hybridization autoradiograms were quantitated by computerized densitometry. Preliminary results indicate a greater expression of angiotensinogen mRNA in circumscribed regions of SHR brain, and that this difference is enhanced after adrenalectomy.

350.19

EFFECTS OF STRIATAL DOPAMINE DEPLETION ON SOMATOSTATIN NEURONS. T.T. Lu*, M. Goldstein, R.H. Roth, F. Baldino, Jr., and A.Y. Deutch. Depts. of Pharmacol. & Psychiat., Yale Univ. Sch. Med., New Haven, CT 06510, NYU Med. Sch., New York, NY, and Cephalon, Inc., West Chester, PA.

Among the intrinsic striatal neurons is a population of cells containing both somatostatin (SOM) and neuropeptide Y (NPY). Previous studies have revealed that depletion of striatal dopamine (DA) results in changes in striatal tachykinin and enkephalin neurons, as reflected by changes in peptide mRNA levels. We have therefore examined the effects of striatal DA depletion on SOM neurons, using immunohistochemical (IHC) and in situ hybridization histochemical (ICSS) methods. Folowing unilateral 6-OHDA lesions of the midbrain DA cell groups, animals were prepared for IHC or ISHH. Striatal DA deafferentation resulted in decreased (18-25%) numbers of SOM-like immunoreactive (SOM-li) neurons (but increased SOM-li fiber staining) in the nuc. accumbens (NAS); ISHH revealed a corresponding decrease in SOM mRNA in the NAS. In contrast, the number of NPY-li neurons in the ventral striatum was increased (40-42%) on the lesioned side; studies of NPY mRNA are in progress. These data suggest that DA regulates ventral striatal SOM neurons, and further suggest that DA may differentially regulate gene expression of two coexistent peptides within striatal neurons.

350.20

CELLULAR LOCALIZATION OF SUBSTANCE P/NEUROKININ A AND NEUROKININ B mRNAs IN THE RAT RETINA. N. Brecha, C. Sternini, K. Anderson and J. E. Krause. Depts. Anat. & Med., UCLA School of Med., LA, CA 90024 & Depts. Anat. & Neurobiol., Washington Univ., St. Louis, MO 63110.

The mammalian tachykinin (TK) peptides, substance P (SP) and neurokinin A (NKA), and neurokinin B (NKB) are encoded by distinct mRNAs derived from separate SP/NKA and NKB genes. Using antibodies directed to the conserved TK C-terminal region as generalized markers for the TK family and immunohistochemistry, numerous immunoreactive cells were identified in both the ganglion cell layer (GCL) and proximal inner nuclear layer (INL) of the rat retina. In situ hybridization histochemistry with ^{35}S-labeled rat SP/NKA- or NKB-encoding antisense cRNAs showed SP/NKA-encoding transcripts in cell bodies distributed to the GCL and proximal INL, and NKB-encoding transcripts in somata located in the GCL. Northern blot analysis confirmed the presence of these mRNAs in retinal extracts. Nuclease protection experiments showed a single NKB-encoding transcript, but multiple SP/NKA-encoding transcripts (gamma-SP/NKA mRNA> beta-SP/NKA mRNA >> alpha SP mRNA). These results demonstrate that both TK genes are expressed in the rat retina. The differential localization of SP/NKA- and NKB-encoding mRNAs in the INL and GCL documents a cellular specific expression of TK-encoding mRNAs in the retina.

Supported by EY04067, NS21937 and SKB Fellowship.

350.21

PREPROTACHYKININ A GENE EXPRESSION IN RAT ENTERIC AND SENSORY NEURONS. C. Sternini, K. Anderson, G. Frantz, J.E. Krause and N. Brecha. CURE & Depts. Med., Anat. & Biol., UCLA School of Med., Los Angeles, CA 90024, & Depts. Anat. & Neurobiol., Washington Univ., St. Louis, MO 63110.

The preprotachykinin (PPT) A gene generates three mature mRNAs: alpha-PPT A mRNA which encodes substance P (SP), and beta- and gamma-PPT A mRNAs encoding both SP and the related tachykinin, neurokinin A (NKA). In this study, we have used in situ hybridization histochemistry with a T7 transcribed [35]S-labeled rat PPT A antisense cRNA that recognizes alpha-, beta- and gamma-PPT A mRNAs to identify the cell bodies in which the PPT A gene is trancribed in the enteric nervous system and sensory ganglia of the rat. PPT A mRNA is localized in neurons in the myenteric plexus of the esophagus and stomach and in both the myenteric and submucosal plexuses of the small and large intestine. In the trigeminal and dorsal root ganglia, intense labeling was observed in small to medium size somata. The distribution of enteric and sensory ganglion cells expressing PPT A mRNA is comparable to the immunohistochemical pattern of SP-like immunoreactivity as shown in cryostat sections immunostained with SP antisera. These results demonstrate that the PPT A gene is transcribed in a subpopulation of enteric and sensory neurons and that the specific mRNA and the final product of RNA translation are localized in the same structures.

Supported by DK38752, NS21937 and UCLA Faculty grant 3873.

350.22

Cellular localization of mRNA by in situ hybridization in cultured nerve muscle contacts. S.A. Berman, S. Bursztajn, K. Tkach and W. Gilbert. Bio. Labs, Harvard Univ., Cambridge. MA.02138.

Acetylcholine receptor (AChR) synthesis is responsive to a variety of neuronal and non-neuronal factors. To determine if a correlation between innervation and AChR mRNA exists, we examined mRNA at a single cell level via in situ hybridization and autoradiography with an α subunit AChR genomic probe obtained from J.P. Changeux. Muscle cells four days after plating were cocultured with spinal cord explant, ciliary neurons or dorsal root ganglia (DRG). 7-8 days into coculture muscle cells within two explant diameters of the spinal cord explant show a higher density of silver grains than muscle cells alone. A graded decrease in the silver grain distribution is observed as distance from the explant increases. A similar gradient in AChR mRNA distribution is not evident when ciliary neurons are used. Instead, the distribution of mRNA is uniform, but at a much higher density than with muscle cells alone. Neither an increased mRNA level nor a gradient is seen when DRGs are used. We hypothesize that coculture with cholinergic neurons results in a localized increase in AChR mRNA.

351.1

HIPPOCAMPAL GLIOSIS IN A GENETIC MOUSE MODEL OF EPILEPSY. J.V. Brigande*, M. D. Albert*, and T. N. Seyfried. (Spon: G. Balkema) Dept of Biology, Boston College, Chestnut Hill, MA 02167.

El (epileptic) mice have an inherited convulsive disorder that resembles temporal lobe epilepsies in humans (T. Seyfried, and G. Glaser, Epilepsia 26: 143, 1985). The seizures in El mice begin spontaneously around 100 days of age and then persist throughout life. The immunocytochemical distribution of glial fibrillary acidic protein (GFAP) was studied in the brains of El and control C57BL/6J (B6) mice at adolescent (43 days) and adult (240-300 days) ages. GFAP was identified with an anti-GFAP polyclonal antibody (obtained from J. Goldman) using a peroxidase-antiproxidase procedure. The brain sections were counter stained with Mayer's hematoxylin. The mean number of GFAP-positive cells in the hippocampal stratum granulosum area dentatae was about 20 fold higher in the El mice (304 stained cells per square mm, N=4 mice) than in the B6 mice (13 stained cells, N = 4 mice) at adult ages. The GFAP-positive cells in El mice also differed qualitatively from those in B6 mice in having enlarged cell somas and thick, elongated processes. The gliotic reaction in the El mice was not associated with an observable neuronal loss. No differences were observed between the El and B6 mice in the cerebellum or cerebral cortex. There were also no major quantitative or qualitative differences in GFAP-positive cells between the El and B6 mice at the younger age. We conclude that the El mouse can serve as an excellent epilepsy model for studying the association between seizure activity and gliosis. (Supported by NIH grants 23355 and 24826).

351.2

TIME COURSE AND BRAIN DISTRIBUTION OF SEIZURE-INDUCED ASTROCYTE HYPERTROPHY IN RATS. W. Moore, N.W. Milgram, M. Khurgel and G.O. Ivy. Div. of Life Sciences, Univ. of Toronto, Toronto, Ontario, M1C 1A4.

Using immunohistochemical techniques, we have shown that patterns of astrocyte hypertrophy (AH) can be used to trace neural systems which undergo abnormally heightened levels of electrical activity (Neurosci. Abs. 13:1265). We now delineate the time course and more detailed brain distribution of this response. Rats were injected systemically with kainic acid (KA); both seizure time and survival time were varied. The brains were processed for immunoreactivity to glial fibrillary acidic protein (GFAP). Increased immunoreactivity to anti-GFAP was apparant in hippocampus (H) and in the outer layers of neo- and paleocortex within 5 hours after onset of seizure activity. At 24 hours, AH was extensive in these regions, and was also present in amygdala, dorsomedial (DM) and laterodorsal (LD) thalamic nuclei and nucleus reuniens (R); the deep layers of neo- and paleocortex were conspicuously devoid of heightened immunoreactivity. By 1 week following 3-5 hours of status epillepticus, H, DM, LD, R and deep layers of pyriform cortex contained necrotic cells. The relative amount and distribution of immunoreactivity was similar at 1 month but was decreased and more restricted at 4 months after the seizures. These results indicate that rapid, progressive and long lasting degenerative changes result from a single incident of seizure activity. Supported by NSERC.

351.3

EARLY MATURATION OF BRAIN DAMAGE AFTER FLUROTHYL SEIZURES. W.A. Kofke, J. Towfighi, J. Graybeal*, K. Houseman*, R.A. Hawkins. Dept. Anesthesia, Milton S. Hershey Medical Center, Hershey, PA 17033

Brain damage was studied in rats following flurothyl seizure (SZ). There were 4 groups: (1) 45 min (SZ), (2) 45 min control, (3) 45 min (SZ) with 2 h recovery, (4) 45 min control with 2 h recovery. Rats were anesthetized with isoflurane/N_2O, paralyzed, intubated and ventilated. After cannulation of the femoral vessels flurothyl was administered (5% for 1 min and 3% for 44 min) via Ethrane vaporizor. SZs were controlled by blood withdrawal. SZ was stopped with IV thiopental 15 mg/kg. Recovered rats were ventilated for 2 h. EEG power spectra were recorded by computer. Substantia nigra was examined for eosinophilic neurons. All sham brains were normal. 45 min status epilepticus produced fine nigral sponginess with 5-10 eosinophilic neurons per lesional area (PLA) on coronal sections thru the center of the lesion. After recovery nigral sponginess was considerably more course, with 20-25 eosinophilic neurons PLA. The size of nigral damage was similar in both groups. The results demonstrated that flurothyl can be given continuously and quantitatively with adequate oxygenation and control of blood gases. Damage apparent by light microscopy after 45 min SZ matures further after SZ has stopped and the convulsant is eliminated.

351.4

SYNAPTOGENESIS IN KINDLING. N. Hawrylak, F.-L. Chang, D. Treacy, K. R. Isaacs, W.T. Greenough, Neur. Beh. Bio. Prog., Coll. Med., Depts. of Psych., and Cell and Struct. Bio. Univ. of Illinois, Champaign, Il. 61820

Kindling has features that replicate certain forms of human epilepsy. The epileptiform activity induced by kindling is characterized by synchronous hyperactivity of neurons and in this respect it is similar to LTP, a model of learning/memory. Chang and Greenough (Brain Res. 309:35, 1984), have demonstrated an increase of shaft and sessile synapses, suggested intermediaries during synaptogenesis, following LTP.

We adopted an in vitro kindling model from Stasheff et al. (1985). Control or kindling stimulation was administered to the CA1 region of 5 pairs of adjacent rat hippocampal slices. We found a significant increase in the density of shaft (110%) and sessile ("stubby") spine (149%) synapses. The total number of synapses increased by only 0.9% which was not significant. The finding is comparable to those obtained with LTP.

These results support the view that kindling and LTP share a similar mechanism of modifying hippocampal circuitry. Our quantitative EM study strongly suggests that synaptogenesis occurs during kindling. These new synapses may participate in feedforward connectivity increasing synchronization.
Supported by Epilepsy Foundation of America and ONR

351.5

Synaptic Reorganization of Mossy Fibers into Inner Molecular Layer in Human Epileptic Fascia Dentata. T.L. Babb, W.R. Kupfer* and J.K. Pretorius* Dept. of Neurology and Brain Research Institute. Univ. of Calif. Los Angeles 90024.

Reactive synaptogenesis ("sprouting") of recurrent excitatory mossy fibers into the inner molecular layer (IML) of fascia dentata (FD) of rats has been demonstrated to be correlated with hyperexcitability in vitro following prior in vivo kainic acid lesions of CA_3/CA_4 of the rat hippocampus (Tauck and Nadler, J. Neurosci. 1985, 5:1016). We used the Timm's sulphide procedure to stain for mossy fiber (MF) distribution and density in surgically-excised human hippocampi (n=13) known to be epileptogenic by prior in vivo recordings of focal electrographic/clinical seizure onsets. Cell counts of Niss1-stained sections demonstrated differing percents of cell loss in CA_3 and CA_4 which we correlated with the densities of increased mossy fibers sprouting into the IML. Controls (n=2) had no cell loss and no MF sprouting. IML light transmission was quantified objectively with an IBAS image analyzer by standardizing the tissue staining density for each of 6 sections/patient, and paired t-tests demonstrated statistical significance in all IML compared to MML of epileptic but not control tissue. There were no significant changes in outer ML or granule cell staining. The cell loss was correlated significantly for CA_3 ($R=.88$; $p<.01$) and a trend for CA_4 ($R=.50$; $p<.10$) with IML mossy fiber reinnervation. These results demonstrate that chronic hippocampal epilepsy may be attributed to abnormal recurrent excitation.
NIH Grant NS02808.

351.6

KAINATE INJECTION INCREASES GAD mRNA LEVELS IN HILAR NEURONS OF THE RAT HIPPOCAMPUS. S. Feldblum[1*], R.F. Ackermann[2], and A.J. Tobin[1,3,4] (SPON: S.R. Snodgrass). Departments of [1]Biology and [2]Neurology, [3]Molecular Biology Institute, and [4]Brain Research Institute, University of California, Los Angeles, CA 90024.

Decreased inhibition has often been suggested as a causal factor in focal seizure disorders. We are using in situ hybridization to investigate this hypothesis in the kainate-induced seizure model of rats. Our preliminary data suggests an increase, rather than the expected decrease in the production of the GABA synthetic enzyme, glutamate decarboxylase (GAD).

Injection of kainic acid (0.5 ug) into the CA3 pyramidal layer of the ventral hippocampus induces local neuronal damage and a long term increase in seizure susceptibility. Following kainate injection, the number of hippocampal neurons containing GAD mRNA did not change, but the hilar cells of the ipsilateral dentate gyrus showed markedly higher levels of GAD mRNA. We are now evaluating the relationship of these increases to the sprouting that follows the destruction of pyramidal cells by kainate.

This work was supported by a grant to AJT from NINCDS (NS-22256) and grants to RFA from NIH (NS-15654, MH-037916) and a DOE contract (DE-AC03-76-SF00012).

351.7

MOSSY FIBER SYNAPTIC REORGANIZATION INDUCED BY REPETITIVE PENTYLENETETRAZOL SEIZURES. G. Golarai*, I. Parada* and T. Sutula, Department of Neurology, Neurosciences Training Program, University of Wisconsin, Madison, WI 53792.

Synchronous perforant path activation and limbic kindling by periodic electrical stimulation induce mossy fiber synaptic reorganization in the hippocampus as indicated by development of supragranular Timm granules in the dentate gyrus (Science 239:1147, 1988). To determine whether synaptic reorganization occurs in other models of epilepsy, the Timm method was used to study the mossy fiber pathway in rats that received pentylenetetrazol (PTZ), a convulsant that blocks GABA-mediated inhibition. Injections of PTZ (24 mg/kg IP) repeated every other day initially produced no behavioral effects, but eventually induced myoclonus followed by generalized seizures. Rats kindled to generalized seizures by PTZ demonstrated prominent supragranular Timm granules, but rats experiencing only myoclonus or no behavioral response to PTZ showed no evidence of mossy fiber synaptic reorganization. Five days after a generalized seizure evoked by a single dose of PTZ (50 mg/kg IP), there was no evidence of synaptic reorganization. As with electrical kindling, the supragranular Timm granules induced by PTZ kindling developed in the absence of overt neuronal damage. The results show that repeated activation of hippocampal pathways during PTZ seizures induces mossy fiber synaptic reorganization and demonstrate that synaptic reorganization also occurs in generalized models of epilepsy.

351.8

MOSSY FIBER SYNAPTIC REORGANIZATION DEVELOPS AND BECOMES PERMANENT IN PARALLEL WITH PROGRESSION OF KINDLING. J. Cavazos, I. Parada* and T. Sutula. Department of Neurology, University of Wisconsin, Madison, WI 53792.

Kindling induces mossy fiber synaptic reorganization as indicated by development of supragranular Timm granules in dentate gyrus (Science 239:1147, 1988). If synaptic reorganization has a role in development of kindling, supragranular Timm granules should develop in parallel with progression of evoked seizures and become permanent when long-lasting susceptibility to kindling is established (after Class V seizures). Rats received either perforant path or amygdala stimulation until 5-10 AD's, or 5, 10, 20, 30, 50 Class V seizures were evoked. Mossy fiber reorganization was classified at 18 hrs after the last seizure as follows: 0-no granules; 1-sparse granules; 2-prominent granules; 3-confluent granules. Timm granules were noted after 5-10 AD's and correlated with the number of seizures. To determine if synaptic reorganization at various stages of kindling was long-lasting, similar groups of rats were perfused 3 months after the last seizure. Supragranular Timm granules persisted at least 3 months after Class V seizures but were diminished 3 months later in the 5-10 AD group. Supragranular Timm granules develop and become permanent in parallel with the progression of kindling. Mossy fiber synaptic reorganization is reversible prior to Class V seizures, and may be a cellular mechanism of kindling.

351.9

ENHANCED ASPARTIC ACID RELEASE FROM HIPPOCAMPAL SLICES OF EPILEPTIC (El) MICE H.J. Flavin, A. Wieraszko, and T.N. Seyfried. Dept of Biology, Boston College, Chestnut Hill, MA 02167.

The Epileptic (El) mouse represents a natural , genetic model of epilepsy . An adult El mouse seizes spontaneously , but seizures can also be induced by twirling the animal by the tail . The release of putative neurotransmitters (Asp , Glu , and GABA) was studied in hippocampal slices from five El mice and five age-matched nonepileptic C57BL/6J (B6) mice . The average number of observed seizures per El mouse was 86 . Sets of hippocampal slices (400 μ thick) were incubated in a series of normal and high potassium (60 mM) buffers either in the presence or absence of calcium . The amount of transmitter released per sample was expressed as a percent of the total release from a given set of slices . For each transmitter , the basal release was subtracted from the potassium stimulated release in the presence and the absence of calcium . These differences were used to characterize the neurotransmitter release in each mouse strain . The data were expressed as the mean ± SEM . No differences were found between the El and B6 mice for the release of Glu (33.0 ± 2.6 / 34.8 ± 4.6) or GABA (25.0 ± 4.6 / 24.1 ± 4.1) . Asp release , however , was significantly higher in the El mice (39.7 ± 3.8) than in the B6 mice (21.4 ± 2.8) ($p < 0.01$) . Thus, enhanced Asp release may be related to seizures . Supported by the Michael P. Walsh Scholarship , and grants from NSF (8604955) and NIH (23355,24826)

351.10

RELEASE OF EXCITATORY AND INHIBITORY AMINO ACIDS FROM HIPPOCAMPAL SLICES IN TETANUS TOXIN CHRONIC EPILEPSY. J.G.R.Jefferys, P.Mitchell*, L.O'Hara*, J.Hardy*, S.Jordan*, M.Lynch*, J.Wadsworth*. St.Mary's Hosp.Med.Sch., London W2 1PG, UK and National Institute for Medical Research, London NW7 1AA, UK.

Injecting a minute dose of tetanus toxin into rat hippocampus causes a chronic epileptic syndrome. This has been attributed to a disruption of the release of the inhibitory transmitter, GABA. Hippocampal slices prepared from such rats during their active seizure phase retain spontaneous "interictal" and seizure-like actvity in vitro (Jefferys, 1986, J.Physiol.373,24P). Here we have prepared slices 10 to 14 days after injection in order to measure the K^+-evoked, Ca^{++}-dependent release of amino acid transmitters into the superfusate. Tetrodotoxin was included to prevent spontaneous activity. We measured the release of endogenous aspartate, glutamate and GABA by HPLC, and of preloaded radiolabeled D-aspartate and GABA by liquid scintillation counting. The effects of K^+, Ca^{++}, experiment number, and injection type were assessed using analysis of variance. Both glutamate and GABA showed a Ca^{++}-dependent, K^+-evoked release, but radiolabelled D-aspartate and endogenous aspartate did not. The release of GABA was impaired by the tetanus toxin treatment.

We conclude that: (1) radiolabelled D-aspartate is not a suitable marker for glutamate as a transmitter; (2) endogenous aspartate does not behave like a transmitter in this experiment; (3) transmitter release is impaired by the minute dose of intra-hippocampal tetanus toxin needed to induce a chronic epileptic focus.

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351.11

PRESENCE OF INTRINSIC EPILEPTIFORM ACTIVITY IN HUMAN BRAIN SLICES. Steven A. Reid and Reinhard A. Palovcik, Departments of Neurological Surgery and Neuroscience, University of Florida, Gainesville, FL 32610.

We report the occurrence of spontaneous, epileptiform activity in isolated slices of human cortex, obtained in the course of surgery for epilepsy. Prior to removal, the location and extent of the epileptogenic area was mapped using arrays of electrodes placed on the cortical surface.

Tissue obtained in the operating room was quickly sliced into 400 micron thick sections and transferred to a slice transport system for removal to the laboratory. We have recorded electrophysiological activity from such slices for periods up to 20 hours following surgical removal from the patient. This tissue is part of a specimen that would normally be resected in the routine course of the neurosurgical procedure.

Slice epileptiform activity consisted of episodes of spontaneous large spikes with characteristics similar to those found on the corresponding corticogram and EEG. Slices from areas neighboring the epileptic focus showed little or no spontaneous activity and never exhibited the large spikes found in the area of the focus. During an epileptiform burst we were able to record extracellular complex spikes from layers 4 and 5 that were as large as 20 mv but were more often between one and five mv.

Evoked activity was recorded from layer five upon stimulation of layers three and four. Evoked spikes ranged from 100 microvolts to 3.65 millivolts and were between 1 and 4 msec. in duration. Evoked spikes could be completely inhibited by iontophoretic application of GABA. Application of a dilute ($5x10^{-6}M$) ferric chloride solution (a known topical epileptogenic agent) potentiated the size of evoked spikes. We have observed a similar potentiation of evoked action potentials in rat hippocampal slices with the application of iron. These findings suggest that relatively small volumes of cortical tissue are sufficient to initiate and maintain epileptic discharges in human focal epilepsy.

This research was supported by the Veterans Administration and University of Florida.

351.12

ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONS IN THE SUPERFICIAL LAYERS OF HUMAN TEMPORAL NEOCORTEX. J.C. Lacaille, G.G.C. Hwa* and M. Avoli. MNI, McGill University and Université de Montréal, Que., CANADA

Intracellular recordings were performed in 34 neocortical neurons in slices of human tissue obtained from temporal lobe epileptic foci (n=9) or brain tumor (n=1). Cells were located within 750 um from the pia. Two neurons were of the fast spiking type but the majority (32/34) behaved like regular spiking neurons. They displayed resting membrane potential of 75 + 1.4mV, (mean + S.E.; n=31), Ri of 32 + 2.4 M (n:31), time constant of 13.8 + 1.1 ms (n=23) and action potential (AP) amplitude of 92+ 1.6 mV (n=30). In most neurons a single AP was followed by a fast afterhyperpolarization (AHP_f), a depolarizing afterpotential and a medium AHP (AHP_m). Following repetitive firing of APs, a slower AHP (AHP_s) also appeared. I-V relationships usually demonstrated inward rectification in the depolarizing direction, depolarizing sag and anomalous rectification. In 14 neurons focal electrical stimulation elicited an EPSP-IPSP sequence but did not induce burst firing. Rhythmic, spontaneous PSPs were observed occasionally (n=3). The properties of these neocortical neurons are similar to those observed in experiments performed in the neocortex of other mammalian species in normal conditions. Supported by MRC, FRSQ and Sloan Found.

351.13

INHIBITORY SYNAPTIC INPUT CORRELATES WITH INTRINSIC MEMBRANE PROPERTIES IN NEOCORTICAL PYRAMIDAL CELLS. L.R. Silva, Y. Chagnac-Amitai and B.W. Connors. Depts. of Neurology, Stanford University, Stanford, CA 94305, and Neurobiology, Brown University, Providence, RI 02912.

Layer 5b of rat SmI cortex has two types of pyramidal cells, as defined by their intrinsic membrane properties: bursting cells and regular-spiking cells. The local synaptic control of these cell types is of particular interest because many layer 5 pyramidal cells are output neurons, and bursting cells may play a key role in epileptic discharge.

Layer 5b pyramidal cells were recorded intracellularly in slices of rat SmI. PSPs were evoked by exciting nearby cells with L-glutamate (GLU) or acetylcholine (ACh) applied focally to the same layer. GLU evoked EPSPs and, often, prominent IPSPs in regular-spiking cells. In contrast, in bursting cells GLU evoked EPSPs but no apparent IPSPs. ACh applied near regular-spiking cells reliably evoked IPSPs (cf. McCormick & Prince, J. Physiol., '86). However, IPSPs evoked by ACh were very rarely observed in bursting cells.

These data demonstrate that: 1) both regular-spiking and bursting cells in layer 5b receive excitatory input from neighboring cells, and 2) regular-spiking cells, but not bursting cells, are strongly inhibited by layer 5 interneurons.

Supported by MH09641, the Klingenstein Fund and NS25983.

351.14

ANATOMICAL AND PHYSIOLOGICAL PROPERTIES OF BURSTING CELLS IN LAYER V_B OF SOMATOSENSORY CORTEX. Y. Chagnac-Amitai*, A. Agmon and D.A. Prince (SPON:K.L. Chow). Dept. of Neurology, Stanford Medical Center, Stanford, CA 94305.

Previous studies have shown a small population (<10%) of intrinsic bursting neurons in layers IV and superficial V of guinea-pig cortex (B.W. Connors et al. J. Neurophys. 48: 1302, 1982). We identified a second group of cells with these properties in layer V_B of brain slices from somatosensory cortex of rodents. Intracellular labelling with Lucifer Yellow and reaction with anti-Lucifer Ab's showed that these neurons are among the largest pyramids in layer V_B. Approximately 50% of the cells impaled at this specific cortical depth display bursting properties.

Spike generation of these neurons is distinguished by prominent DAPs, and directly evoked bursts of action potentials which are not blocked at membrane potentials positive to rest. More than one burst or repetitive doublets are generated during a depolarizing current pulse. Synaptic responses are characterized by a prolonged voltage-dependent depolarizing component and often by an absence of a visible fast IPSP.

TTX blocked all spiking activity and the underlying intrinsic depolarizations, while several Ca^{2+} blockers failed to block the burst generation. We speculate that layer V_B bursting pyramidal neurons function as amplifiers of cortical output under normal and epileptogenic conditions.

Supported by NIH grant NS12151 and the Pimley Fund.

351.15

FURTHER STUDIES OF THE SUSTAINED DEPOLARIZATION OF SEIZURE-LIKE DISCHARGES IN IMMATURE CA3 HIPPOCAMPAL NEURONS. J.W. Swann, K.L. Smith, C. Yu. Wadsworth Ctr. for Labs & Res., NYS Dept. of Health, Albany, NY 12201

During prolonged (10-30 sec.) seizure-like discharges individual pyramidal cells undergo a sustained depolarization (SD). Synchronized afterdischarges ride the envelope of this depolarization. Previously we have shown that the SD is a separate physiologic process from the synchronized discharges and the product of localized synaptic interactions. To further examine its synaptic nature we have begun recording intracellularly with Cs microelectrodes. Whenever Cs is injected intracellularly a dramatic and unexpected 3-5 fold increase in the amplitude of the sustained depolarization is produced. Under current clamp conditions the enhanced SD uniformly reverses polarity near OmV. The amplitude of the SD is a monotonic function of membrane potential. These latter observations are consistent with the notion that the SD is a synaptic event.

Computer simulation using a membrane parallel conductance model predicts that if Cs blocks K+ conductances that are concurrent with excitatory synaptic currents a nonlinear increase in the amplitude of epsps occurs. Thus our results also suggest that a K+ conductance(s) plays an important role in limiting the degree of intracellular depolarization during seizure discharges. (Supported by NINCDS grant NS18309)

351.16

PAIRED INTRACELLULAR RECORDINGS REVEAL MONO- AND POLYSYNAPTIC EXCITATORY INTERACTIONS IN IMMATURE HIPPOCAMPUS. K.L. Smith*, J. Turner and J.W. Swann (SPON: W. Shain). Wadsworth Ctr. for Labs and Research, NYS Dept. of Health, Albany, NY 12201.

Isolated segments of CA3 subfield were taken from 10-15 day old rats. Epileptiform activity was induced by bath application of 1.7 mM penicillin. When depolarizing current was applied to single neurons, (both bursting and fast spiking) 60% of these cells initiated epileptiform discharges. Once a cell initiated seizure-like discharges a second cell was impaled. Synaptic interactions occurred in over 50% (16/30) of these pairs. One pair was electrically coupled. Two pairs had monosynaptic epsps and three pairs interacted in both directions. One cell produced a monosynaptic epsp with an amplitude of 3.14mV, a latency to onset of 1.23 msec, a latency to peak of 7.2 msec and a probability of 92%. In several respects our results differ from those obtained in the mature hippocampus. The most striking difference is the higher frequency of synaptic interaction in paired neurons. We suggest early development of local circuits is characterized by an enhanced degree of synaptic interaction. Such a maturational difference may explain why the immature hippocampus is more susceptible to seizure-like activity. The anatomical correlates of these synaptic interactions are under investigation. (Supported by NS18309; RR02984)

351.17

MOSSY FIBER LESION DOES NOT PROTECT CA3 HIPPOCAMPAL PYRAMIDAL CELLS FROM KAINIC ACID BY REDUCING KAINATE RECEPTOR DENSITY. J.P. Vicedomini, M.M. Okazaki and J.V. Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Intracerebroventricular (i.c.v.) administration of kainic acid (KA) to rats provokes limbic seizure activity associated with neuronal degeneration. CA3 hippocampal pyramidal cells are exceptionally vulnerable to i.c.v. KA. Previous studies demonstrated that the vulnerability of these neurons depends upon an intact mossy fiber innervation, because a prior lesion of this pathway protected the denervated CA3 pyramidal cells from destruction. A mossy fiber transection was effective immediately, within 10 min, suggesting that the protective effect resulted from cessation of impulse flow along the mossy fiber pathway. The present study tested an alternative hypothesis: namely, that the mossy fiber lesion led to a rapid down regulation of kainate receptors in area CA3.

The mossy fiber pathway of the rostral hippocampal formation was transected unilaterally with a Scouten wire knife and the rats were sacrificed 10 min, 2 h or 6 d later. To obtain autoradiographic images of kainate receptor binding, unfixed brain sections were incubated with [^{3}H]KA and apposed to ^{3}H-sensitive Hyperfilm for 4 weeks.

In agreement with Represa et al. (Neuroscience, 20, 739 (1987)), the mossy fiber lesion substantially reduced kainate receptor binding in stratum lucidum of area CA3 within 6 d. The reduction was observed only within that region of the rostral hippocampal formation in which CA3 pyramidal cells had been extensively denervated. Only modest effects of the transection, at most, were evident at the 10 min or 2 h survival times. Thus a rapid down regulation of kainate receptors probably does not account for the neuroprotective effect of a mossy fiber lesion. Protection most likely results from the interruption of impulse flow. This effect of the lesion might: (1) prevent KA from releasing a potentially damaging substance from the mossy fibers and/or (2) abolish a postsynaptic interaction between KA and one or more neuroactive substances released by the mossy fibers. (Supported by NIH grant NS 17771.)

351.18

SYNAPTIC INHIBITION AND THE PRODUCTION OF ICTAL DISCHARGES BY CHOLINERGIC AND ANTICHOLINESTERASE AGENTS IN RAT HIPPOCAMPUS. *F.J. Lebeda, T.H. Ton*, and P.R. Rutecki.* Sect. of Neurophysiol., Prog. in Neurosci., Dept. of Neurol., Baylor College of Medicine, Houston, TX 77030.

A variety of convulsants (*e.g.*, picrotoxin (PTX), 4-aminopyridine) produce brief (20-200 ms), synchronous, repetitive discharges in hippocampal slices whether or not the agent interferes with GABA-mediated inhibition. This study was conducted to determine the role of GABAergic transmission during the epileptiform activity induced by partial (bethanechol, oxotremorine and pilocarpine) and full (acetylcholine, muscarine and carbachol) muscarinic agonists (1-150 μM) as well as irreversible anticholinesterases (anti-ChEs; DFP or soman; 10-25 μM).

Besides producing brief (interictal) events within 30 min of exposure in the CA3 subfield, prolonged exposure (1-2 h) to these agents could produce repetitive ictal discharges — extracellularly recorded events lasting >2 s. A transition from interictal to ictal discharges also occurred within 30 min with the co-application of salines containing 7.5 mM K^+ or 5-10 mM Li^+. The ictal discharges were blocked following washout of the test agent or by the co-application of atropine (0.5-10 μM). The ictal events induced by the partial muscarinic agonists or by the anti-ChEs were converted to interictal discharges by the co-application of 10-50 μM bicuculline or PTX. In contrast, higher concentrations of PTX (200-500 μM) were required to convert the ictal events induced by full agonists. The amplitudes of the intracellular correlates of both the interictal and ictal events reversed in polarity at relatively negative potentials (*ca.* −15 mV). These results suggest that inhibitory neurotransmission plays a role in the generation of the interictal and ictal events induced by agents that modify cholinergic neurotransmission. (Supported by USAMRCD DAMD17-86-C-6029, AFOSR 85-0178, NIH grants NS 11535 and 01049, and the Klingenstein Fund.)

351.19

NORADRENERGIC MODULATION OF EPILEPTIFORM ACTIVITY IN THE HIPPOCAMPUS. *P.A. Rutecki, T.H. Ton*, F.J. Lebeda.* Sect. of Neurophysiol., Dept. of Neurology, Program in Neurosci., Baylor Col. of Med., Houston, TX 77030.

Noradrenergic agonists have been characterized as having both pro- and anticonvulsant effects. We have examined the effects of norepinephrine (NE) and selective α- and β-adrenergic receptor agonists and antagonists on the frequency of epileptiform discharges that were recorded extracellularly in the CA3 region of rat hippocampal slices. The discharges were produced by the bath application of saline containing 10 μM picrotoxin (PTX) or 7.5 mM extracellular potassium (high K^+).

In high K^+ saline, coapplication of 5 μM NE increased the frequency of spontaneously occurring discharges by 37% while 30 μM NE slowed the frequency by 60%. The PTX-induced discharge frequency was significantly slowed by 10-30 μM NE (26-47%); however, at low NE concentrations there was not a statistically significant increase in frequency. In both model convulsants, isoproterenol (0.1-1 μM), a β agonist, significantly increased the frequency. The increase in frequency induced by NE or isoproterenol was blocked by timolol (0.1-1 μM), a β receptor antagonist. Timolol, however, did not block the slowing in frequency produced by 10-30 μM NE. Conversely, the frequency suppression seen with higher concentrations of NE was blocked by the α antagonist phenoxybenzamine (10 μM). Phenoxybenzamine also revealed a significant frequency enhancement of 1-10 μM NE for PTX-induced discharges (30-84%).

These results demonstrate a concentration-dependent effect of NE on the frequency of high K^+- and PTX-induced epileptiform discharges *in vitro*. At low NE concentrations the increase in frequency appeared to be a β-adrenergic effect, and the decrease in frequency seen at higher NE concentrations appeared to be mediated by α-adrenergic receptor activation. (Supported by the Klingenstein Fund, USAMRDC DAMD 17-86-C-6029, AFOSR 85-0178, and NIH grants NS11535 and NS01049.)

NEUROTOXICITY IN DEVELOPMENT II

352.1

INTERACTION OF ETHANOL WITH PHOSPHOINOSITIDES DURING BRAIN DEVELOPMENT. L. G. Costa and W. Balduini*. Dept. of Environmental Health, University of Washington, Seattle, WA 98195.

The developmental profile of muscarinic receptor (MR)-stimulated phosphoinositide (PI) metabolism in rat brain is strikingly similar to that of the rat brain growth spurt (JPET 241:421, 1987). This has led to the hypothesis that the enhanced hydrolysis of membrane PIs by cholinergic agonists during this period may have a relevant role in cell proliferation and differentiation. We have investigated whether exposure of rat pups to ethanol (ETOH) during the brain growth spurt (4 g/kg/day from day 4 to day 10) would alter MR-stimulated PI metabolism in cerebral cortex slices. ETOH caused long lasting microencephaly but did not alter the pups' body weights compared to an equally-handled, sucrose fed group of animals. MR-stimulated PI metabolism was significantly reduced in ETOH-exposed rats on day 7 but not on days 12, 20 and 45, when microencephaly was present. A similar treatment in adult rats did not cause these alterations despite similar blood ETOH concentrations (approx. 250 mg/dl). In vitro experiments indicated that ETOH (150-300 mM) inhibited MR-stimulated PI metabolism in cerebral cortex from 7 day-old, but not adult, rats. These results confirm that the developing brain is particularly sensitive to the effects of ETOH and suggest that the PI system coupled to MR could represent a relevant target for its neurotoxicity. (Supported in part by a grant from ADAI, Univ. of Washington and by NIEHS grants ES-04696 and ES-03424).

352.2

SYNAPTOTOXIC EFFECTS OF CHRONIC PERINATAL ETHANOL EXPOSURE DEMONSTRATED In Vivo. J.L. Walewski*, M. Okamoto and R.L. DeGrandchamp* Dept. of Pharmacology, Cornell Univ. Medical College; New York, N.Y. 10021 and Neurotoxicology Lab, Rutgers Univ.; New Brunswick, N.J. 08903

We postulate that low-level Chronic Perinatal Ethanol Exposure (CPEE) selectively affects some processes involved in synapse formation. We report an animal model demonstrating the synaptotoxic effects of CPEE, using an ETOH dose below the threshold for Fetal Alcohol Syndrome. ETOH 3% v/v mixed with high-protein liquid diet (HPLD) (Bio-Serve) was the only diet source. The controls received isocaloric sucrose (HPLD). ETOH R_x began on day 8 of pregnancy. ETOH 3% v/v did not sig. reduce the body weights and diet consumption of the dams, nor the gross growth of the ETOH pups vs. controls. Blood ETOH conc., determined by NAD-NADH assay, was approx. 50 mg % in both the dams and pups throughout the treatment. Standard neuromuscular twitch preparations utilizing the sciatic nerve-gastrocnemius muscle, were done on 1,2 and 3 week old pups in vivo. The physiologic and pharmacologic functional tests indicated that: [1] the ETOH pups had a longer rise time of single twitch contraction (2 wk., 120% ; 3 wk., 122% of control). This disappeared with direct muscle stim. in a curarized prep., indicating that the deficit is neuronal. [2] 75 Hz. indirect stim. revealed faster tetanic fatigue by ETOH : T1/2 for C = 9.41 +/- 1.83 sec. vs. E = 4.51 +/- 0.23 sec.. [3] Physostigmine twitch potentiation in 3 wk. ETOH pups, was enhanced approx. 2 fold.

Light microscopic endplate morphology revealed cholinesterase and neurofilaments in situ. In the 2 and 3 wk. ETOH pups, the terminal branches were swollen and their number reduced; while endplate length was shortened by 16.3% and 23%, respectively. These data indicate a selective synaptic pathology by CPEE. (Supported by DA-00591, NIDA)

352.3

IBOTENIC ACID INDUCED DEMYELINATION AND INFLAMMATORY RESPONSE. P.J. Coffey*, V.H. Perry* and J.N.P. Rawlins* (SPON: D. Gaffan). Dept. of Experimental Psychology, Oxford University, England.

Demyelination induced by ibotenic acid within the central nervous system coincided very closely with the borders of aregion containing a high density of leucocytes (Coffey et al., Neurosci Letts., 84: 178, 1988). To assess further the correlation between the extent of the recruited haemopoietic cells and demyelination after ibotenic acid injections, rats were exposed to either 700 rads, 900 rads, 900 rads with a lead shield covering the head or no irradiation, and were then given ibotenic acid injections (0.2µl of 10µg/µl) into the medial septum. The number of neuronal cells, non-neuronal cells and those expressing the CR3 antigen (microglia, macrophages and neutrophils), as revealed by OX42 immunocytochemistry, were counted in the region of the lesion. Demyelination was measured by an independent observer in ajacent sections. This study showed that there was concomitant reduction in demyelination and cell recruitment after exposure to irradiation, but no change in neuronal loss. These results support the view that demyelination following ibotenic acid injections is due to a non-specific or "bystander" effect of the inflammatory response. The integrity of the blood-brain barrier will also be discussed.

352.4

IMMUNE SYSTEM RESPONSE TO STERILE LESIONS IN RAT BRAIN. H. Akiyama*, S. Itagaki*, P.L. McGeer, and E.G. McGeer (SPON: D.D. Greenwood). Kinsmen Lab, Dept Psych, Univ of B C, Vancouver, Canada, V6T 1W5.

Expression of major histocompatibility complex (MHC) class I and class II surface glycoproteins was observed immunohistochemically in rat brain following sterile lesions. These were induced by epidural kainic acid application, lateral hypothalamic 6-hydroxydopamine injections, or intracortical needle cuts. Round to ameboid cells expressing both class I and class II MHC antigens as well as leukocyte common antigen (LCA) were observed quickly to invade the necrotic central area of the lesion. In areas peripheral to the necrosis, microglia became reactive. These microglia displayed MHC class I antigens as early as 1-2 days after lesioning, and class II antigens at 4-5 days. Class I expressing microglia outnumbered class II but many expressed both MHC classes and were also positive for LCA. MHC expression peaked in 1-2 weeks and gradually declined thereafter. After 3-6 months, the presence of these surface markers on microglial cells had almost disappeared. Reactive astrocytes proliferated near the lesion site and were still prominently expressing glial fibrillary acidic protein after 6 months. They were not observed to express either class I or class II MHC antigens. Round cells positive for the PAN-T lymphocyte marker were also observed in the lesioned area suggesting a significant role for T lymphocytes.

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352.5

NEONATAL EXPOSURE TO THE LIMBIC-SYSTEM NEUROTOXIN, TRIMETHYLTIN (TMT), IMPAIRS WORKING MEMORY IN PREWEANLING RATS. C.V. Pickens* and M.E. Stanton (SPON: M.I. Gage). Northrop Services, Inc. and Neurotoxicology Division, U.S. EPA, Research Triangle Park, NC 27711.

Neonatal exposure to TMT causes a loss of hippocampal pyramidal cells and impairs cognition in adult animals. Relatively little is known, however, about the effects of neonatal TMT on cognition in infant animals. Here we report that neonatal TMT administration prevents delayed-alternation learning in 18-day-old rat pups.

Long-Evans rat pups received an i.p. injection of either TMT (6 mg/kg) or saline vehicle on postnatal day ten (PND 10). These pups were then tested on PND 18 for their ability to learn two tasks in a T-maze. One task was discrete-trials-delayed-alternation (working memory) and the other was a simple spatial discrimination (reference memory). Testing occurred on PND 18 because this appears to be the age at which normal rat pups can first perform the alternation task (see Green and Stanton, Behavioral Neuroscience, in press, for results and methodological details). TMT transiently impaired learning, but not assymptotic performance, of spatial discrimination, but utterly abolished learning of delayed alternation.

These results indicate that TMT can be shown to impair cognition during development; and suggest a role for limbic system maturation in the ontogeny of working memory.

352.6

ENRICHED REARING ATTENUATES PERFORMANCE DEFICIT INDUCED BY NEONATAL MONOSODIUM GLUTAMATE (MSG) INJECTIONS. S. Fong*, M.J. Saari, J. Armstrong*, and A. Shivji. Neuroscience Research Unit, Nipissing Univ. Coll., North Bay, Ontario, P1B 8L7.

Recovery of function following some brain lesions can be influenced by the post-lesion rearing environment. We report here that impaired performance in the place navigation task following neonatal MSG injections is also sensitive to the consequent rearing environment.

Male Wistar rat pups were cross fostered at birth and injected daily with MSG (4g./kg.) on days 2 to 12 after birth. Control pups received an equivalent volume of saline. Following weaning at 25 days the rats were randomly allocated to enriched or isolated rearing to yield 4 treatment groups (MSG-Enriched; MSG-Isolated; Saline-Enriched; Saline-Isolated).

After 35 days in their respective rearing environments all rats were weighed and tested in an open field. Activity (grid crosses) and rearing were recorded in an open field. The rats were then tested using the place navigation task.

Multivariate analysis of variance of the mean daily latency in the place navigation task, open-field activity and rearing and body weight measures revealed a significant two way interaction of the rearing environment by MSG-Saline manipulations. Univariate F tests and associated multiple comparisons showed that the MSG-Isolated rats were profoundly impaired in the place navigation task while the MSG-Enriched rats performed nearly as well as saline injected controls. The weight measure also revealed a significant univariate F for the interaction. The open-field measures contributed significant main effects for rearing environment (the isolated rats reared less than enriched) and that neonatal MSG treatment increased open-field activity.

352.7

NEOVASCULARIZATION IN THE KAINIC ACID-INDUCED LESIONS OF RAT STRIATUM. AN IMMUNOHISTOCHEMICAL STUDY WITH LAMININ. K. Shigematsu*, H. Kamo*, I.Akiguchi*, M. Kameyama*, H. Kimura*, P.L. McGeer* and E.G. McGeer* (SPON:H. Ando). Dept. of Neurology, Faculty of Medicine, Kyoto University, Kyoto 604, JAPAN.

Vascular changes that follow stereotaxic injection into the rat striatum of kainic acid, excitatory neurotoxin, were studied at different time intervals after the lesion by laminin immunohistochemistry. 2 µg of kainic acid was dissolved in 1 µl isotonic saline and was injected into the striatum of male rats weighting approximately 300g of the Wistar strain. At various times after treatment (2, 7, 28 days and 3 months) the animals were sacrificed by transcardial perfusion with a fixative containing 4% paraformaldehyde under deep anesthesia. The brains were removed and sections 20 µm thick were prepared on cryostat. Tissues were stained for laminin, tyrosine hydroxylase(TH) and glial fibrillary acidic protein(GFA) using standard immunohistochemical procedures. Immunohistochemistry using antibodies against laminin, a basement membrane glycoprotein, revealed striking neovascularization in the lesioned striatum. The increased laminin-immunoreactivity remained at least up to 3 months after the operation. Rapid and profound changes in striatal astrocytes were demonstrated by increased GFA-immunoreactivity, whereas TH-immunoreactive axons in the injected region did not markedly differ from the control.

352.8

LONGTERM EXPOSURE TO PCBs ALTERS DOPAMINE CONCENTRATIONS IN MONKEY NEOSTRIATUM. R.F. Seegal, K.O. Brosch*, R. Okoniewski* and B. Bush*. Wadsworth Labs, N.Y.S. Dept. of Health, Albany, NY 12201.

PCBs decrease striatal dopamine (DA) concentrations in laboratory rodents (Agrawal et al., 1981; Seegal et al., 1986). However, doses used were greater than those that induce behavioral change in monkeys (Bowman et al., 1978; 1981).

To determine whether low-dose PCBs alter DA function in a species neurochemically similar to humans, we exposed adult male Macaca nemestrina (pig-tailed macaque) daily to corn-oil (controls) or corn-oil + PCBs (Aroclor 1260, 800 to 3200 ug/kg/day) for 26 weeks. Monkeys were then deeply anesthetized and sacrificed by overdose. Brains were then removed, chilled in saline, frozen in dry-ice and stored at -80°C.

Coronal 2-mm sections corresponding to plates A23 to A6 of Winter et al. (1969) were cut. Two-mm punches (mean wet weight = 8 mg) from the caudate (CA), putamen (P) and globus pallidus (GP) were homogenized in ice-cold perchloric acid + EGTA. Supernatants were analyzed by HPLC.

Monkeys remained healthy and active throughout PCB exposure. No signs of toxicity were noted. However, PCB exposure significantly altered neostriatal DA concentrations: DA concentrations increased in CA and P at the two middle doses and decreased in GP at all doses. In addition, DOPAC/DA ratios decreased, indicative of decreased neuronal activity.

Thus, exposure to PCBs alters DA-ergic function in primate neostriatum. These results may explain movement disorders in chlorinated hydrocarbon exposed humans (Klawans et al., 1987). Supported in part by NIEHS Grant #3884.

352.9

POLYCHLORINATED DIBENZOFURANS ALTER CENTRAL BIOGENIC AMINE FUNCTION IN ADULT RAT. K.O. Brosch*, R.F. Seegal and B. Bush*. (SPON: D. Martin). NYS Dept. Health, Albany, NY 12201.

Polychlorinated biphenyls (PCBs) are often contaminated with the more toxic polychlorinated dibenzofurans (PCDFs). Furthermore, municipal waste incinerators produce significant levels of PCDFs (Rizzardini et al.,1983). We recently demonstrated (NSA, 13:933, 1987) that PCDFs, at concentrations as low as 2 ppb, are capable of altering in-vitro catecholamines in pheochromocytoma (PC-12) cells. In order to determine whether similar changes take place in an intact mammalian preparation, we exposed laboratory rodents to PCDFs alone or in combination with PCBs.

PCDFs were separated from PCBs by Florisil-column chromatography; the separation was validated using GC-MS. Adult male Wistar-derived rats were given ad-lib access to adulterated chow containing 1000 ppm PCBs and/or 20 ppb PCDFs (equivalent to the PCDF contamination found in the original PCB mixture) for 28 d. Following exposure, the animals were sacrificed, their brains rapidly removed, dissected and homogenized in ice-cold perchloric acid + EGTA. Biogenic amines and metabolites were analyzed by HPLC.

Exposure to either PCBs or PCDFs significantly decreased metabolite to neurotransmitter ratios (biochemical measures of neuronal activity). In striatum and lateral olfactory tract DOPAC/DA and HVA/DA were decreased; in hippocampus, hypothalamus and brainstem 5-HIAA/5-HT ratios were decreased.

These in-vivo results confirm earlier in-vitro findings and suggest that PCDFs may prove to be neurotoxins of significant public health concern.

Supported in part by EPA Grant #8138.

352.10

AN EVALUATION OF GLUTAMATE INVOLVEMENT IN THE DEVELOPMENT OF THE SOMATOTOPIC ORGANIZATION OF THE NEOCORTEX OF THE RAT. Karl F. Jensen, Neurotoxicology Division, U.S. EPA, Research Triangle Park, NC 27711

Neonatal administration of glutamate dramatically alters the development of the visual and auditory systems. To evaluate its effect on the developing somatosensory system, 4mg/g was administered daily from PND-0 to PND-5. On PND-7 the animals were anesthetized, perfused and the cortex was removed, flattened, tangentially sectioned and reacted for cytochrome oxidase (CO) activity. The somatotopic pattern of CO activity in glutamate treated animals appeared normal. In additional animals, CPP, a potent glutamate antagonist, was administered daily (4mg/kg) from PND-0 to PND-5. The somatotopic pattern of CO activity in CPP treated animals appeared normal. The observation that neither glutamate excitotoxicity nor glutamate antagonism alters development of somatotopic organization suggests that, in contrast to the visual and auditory systems, glutamate does not play a major role in patterning afferents in the somatosensory system.

352.11

EFFECTS OF METHYLMERCURY ON EXCITATORY AMINO ACID RECEPTORS IN THE MOUSE CEREBRUM. B. H. Choi, J. Lincoln*, P. Amodei* Neuropathology, Univ. of Calif. Irvine, Irvine, CA 92717.

Methylmercury (MeHg) is a highly neurotoxic environmental pollutant that causes selective damage to neurons of the calcarine cortex and precentral gyrus of the cerebrum and granule cells of the cerebellum in adults. However, the precise mechanism of neurotoxic damage or selective vulnerability of specific neuronal groups in MeHg poisoning remains unclear. In order to investigate the role of excitatory amino acid transmitter pathways in neurotoxic effects as well as selective neuronal damage in MeHg poisoning, we have examined the status of the N-methyl-D-aspartate (NMDA) kainate (KA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in brains obtained from MeHg poisoned young adult mice and controls by the use of in-vitro radioautographic methods, and analyzed with a microcomputer-assisted imaging system.

A significant increase in density of both AMPA and KA was observed in the parietal cortex of MeHg groups as compared to controls. Also noted was a significant decrease in NMDA density in the frontal cortex of MeHg groups. This modification in receptor density may represent excitotoxic damage and/or plastic changes following MeHg poisoning.

These results suggest that one of the pathways of MeHg neurotoxic damage may be mediated through excitatory amino acid transmitter systems.

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352.12

EFFECTS OF KAINIC ACID LESIONS ON AFFERENT INPUTS TO THE LATERAL SEPTAL REGION. P.W. Nance and D. M. Nance. Depts. of Medicine and Anatomy, Dalhousie Univ., Halifax, N.S., B3H 4H7, Canada.

Kainic acid (KA) lesions of the lateral septal area block the anterograde transport of anatomical tract tracers from the lateral septal region, but have minimal effect on the retrograde transport of tracers from the lesion site (Hopkins, et al, *N.S. Abst*,**10**: 423,1984). This is consistent with the concept that toxic lesions destroy cell bodies whereas fibers of passage and axon terminals are spared. The septal area receives a variety of neurochemically specific inputs and among these, leucine enkephalin, substance P, serotonin, and neurotensin have been localized in terminals of the lateral septal region. To examine further the specificity of KA, unilateral or bilateral KA lesions were produced in the lateral septal area of rats and following a 3-10 week survival period, animals were perfused with 4.0% paraformaldehyde. Sections (40 µm thick) were agitated overnight in rabbit antibodies to either leucine enkephalin (1/10000), serotonin (1/3000), substance P (1/10000) or neurotensin (1/7500) (ImmunoNuclear, Stillwater, MN) containing 1.0% normal goat serum and 1.0% Triton-X 100 in PBS. The immunoreactivity was visualized using either the ABC technique (Vector Labs.) or PAP technique (Cappel) with DAB as the chromogen. Localization of leucine enkephalin-, substance P-, neurotensin- and serotonin-like immunoreactivity in the septal region following a KA lesion indicated that all four transmitters were present in axons and terminals within the lesion sites and the staining appeared more intense than on the unlesioned side of the septum or relative to unoperated rats. The immunoreactive fibers were compressed as though a similar number of afferent fibers were distributed throughout a much smaller neuropil. This apparent constriction of numerous axons and terminals within the compressed KA lesion site was similar for all four transmitters. Thus, KA has a toxic effect on cell bodies within the lateral septal area, but spares chemically specific afferent fibers and terminals. Supported by M.R.C. of Can.

352.13

THE EFFECTS OF ASPARTAME INGESTION ON THE DEVELOPMENT OF THE VISUAL SYSTEM IN GUINEA PIGS. D.A. Burstein*, T.H. Milhorat, L.A. Scribani *and D.L. Dow-Edwards. Laboratory of Cerebral Metabolism, Dept. of Neurosurgery, State University of N Y, Health Science Center at Brooklyn,450 Clarkson Ave., Brooklyn, N.Y. 11203.

Aspartame (Nutrasweet®) is commonly used by pregnant women as an artificial sweetener. Once ingested, aspartame is metabolized into phenylalanine, aspartic acid and methanol. Methanol is known to be toxic to the visual system in the adult guinea pig and human. Therefore, we have investigated the toxicity of aspartame with regard to the developing visual system in the guinea pig.

Virgin Duncan-Hartley guinea pigs were time mated in our laboratory. We administered aspartame at 500, 250, or 0 mg/kg body weight in sesame oil by mouth between day 1 of gestation and day of birth. A non-treated control group was also maintained.

At the 55th day of gestation some animals were sacrificed, fetal orbits were removed, fixed in buffered formalin, and sectioned for histopathological analysis. Thickness of retinal layers and alterations of cellular morphology were quantified. Other animals were allowed to give birth and the offspring were evaluated at 30 days of age for functional activity of brain structures within the visual system using the quantified deoxyglucose method (Sokoloff).

The results of the histopathological studies and brain functional activity will be correlated.

Supported by NIH Grant NS22766

352.14

DEVELOPMENTAL TOXICITY OF ASPARTAME IN THE GUINEA PIG **D.L. Dow-Edwards, L.A Scribani*, L.A Freed* D.E Adler* and T.H Milhorat; Laboratory of Cerebral Metabolism, Dept. of Neurosurgery, State University of N.Y., 450 Clarkson Avenue, Brooklyn, N.Y. 11203.**

Aspartame (eg Nutrasweet ®) is an artificial sweetening agent which is widely used by pregnant women. Previous reports have found developmental toxicity in rats only at very high doses. However, the guinea pig may be a better model for human toxicity than the rat.

Virgin Duncan-Hartley guinea pigs were time mated in our laboratory and assigned to recieve either 500 or 250mg aspartame/kg body weight in a sesame oil vehicle or the vehicle alone by mouth between day 1 of gestation and the day of birth. A nontreated control group was also maintained. On the day of birth pups were weighed, sexed and allowed to remain with their natural mother.

At 15-16 days of age, odor aversion testing was carried out using LiCl. At 30 days of age, brain glucose metabolic activity was quantified using the deoxyglucose method of Sokoloff et al. (J. Neurochem. 28: 897, 1977).

Deficits in odor aversion testing were found to be correlated with altered patterns of brain glucose metabolism in the exposed offspring compared to the controls.

Supported by NIH grant NS22766

TRANSPLANTATION: STRIATUM II

353.1

DISSOCIATED NIGRAL CELL SUSPENSIONS GRAFTED TO WEAVER STRIATUM: A POSSIBLE ROLE FOR HETEROTOPIC DOPAMINERGIC DENDRITES? **L. C. Triarhou, P. Brundin,* G. Doucet, A. Björklund and B. Ghetti. Dept. of Pathology (Neuropathology) and Program in Medical Neurobiology, Indiana Univ. Sch. of Med., Indianapolis, IN 46223 and Dept. of Medical Cell Research, Univ. of Lund, S-223 62 Lund, Sweden.**

Nigral cell suspensions prepared from the ventral midbrain of normal mouse embryos were implanted to the striatum of adult weaver mutants. Prior to perfusion, which was carried out 80 days after grafting, host striata were deprived of the intrinsic dopamine (DA) projection through local injection of 6-OHDA into the ipsilateral substantia nigra (SN). Grafts contained 100-700 tyrosine hydroxylase (TH) immunoreactive neurons. TH axon terminals were found in synaptic contact with unlabeled spines, dendrites and somata in the grafted striatum after 6-OHDA lesions of the SN, providing evidence that innervating axons originated in the graft. TH dendrites extended from the grafts into the striatum as well; they received synaptic input from unlabeled axon terminals; further, they were apposed to unlabeled neurons of the host. In the heterotopic position DA dendrites may, through the release of DA, participate in homologous physiological functions, such as regulation of the activity of grafted DA and non-DA SN neurons and of the release of transmitter from afferent axons to the graft; one may speculate that, in addition, DA dendrites could serve heterologous functions, such as modulation of the activity of corticostriatal fibers and influence of the physiological state of host striatal neurons.

353.2

RECOVERY OF LEARNING AND MOTOR DISFUNCTIONS BY STRIATAL FETAL BRAIN TRANSPLANTS IN PREVIOUSLY LESIONED ADULT RATS. A.L. Piña-Hernández,* H.Fernández-Ortega*, M. Gómez-Chavarín*, and F. Bermúdez-Rattoni. Instituto de Fisiología Celular, UNAM. P.O. Box 70-600. 04510 México.

In this study we demonstrate that fetal striatum grafts can induce recovery of a previously disrupted retention of passive avoidance (PA) and normal motor activity. Male wistar rats were divided into an unoperated control group and a group that sustained large electrolytic lesions in the striatum (ST). All animals were trained to avoid foot shocks in a shuttle box, followed by three retention trials, they were also measured for motor-activity on stoelting boxes. Lesioned animals were divided in two groups, one group received fetal striatal grafts (STG) and the other remained as a lesioned control group (STLx). Eight weeks later all the measurments and training were repeated as mention above.Behavioral results pretransplant showed that striatal lesions produced a significant disruption on PA retention,and enhancement of motor activity. Postransplant results showed a significant improvement of the PA retention and motor activity for the STG but not the STLx group as compared with the unoperated control group. Histological (Nissl) and acetylcholinesterase histochemistry analysis revealed that the ST grafts were well integrated into the host ST, but with less acetylcholinesterase reaction than the host ST tissue. These results support the hypothesis that the fetal brain transplants can restore motor and cognitive disfuctions.

353.3

CONTRALATERAL GRAFTS OF FETAL SUBSTANTIA NIGRA, INDUCE A DECREASE IN ROTATIONAL BEHAVIOR IN NIGRO-STRIATAL LESIONED RATS. J.L. Mendoza-Ramírez*, R. Aguilar-Roblero*, J. Vera* A. Zainos-Rosales*, R. Drucker-Colín. (SPON: H. Brust-Carmona). Depto. de Neurociencias, Instituto de Fisiología Celular, UNAM. México.

This study determined whether fetal substantia nigra (SN) grafts placed on the lateral ventricle contralateral to the lesion, produces improvement in the behavioral deficits produced by unilateral substantia nigra lesions. 10 Rats were injected with 8 ug/4ul of 6-OHDA into the medial forebrain bundle (MFB). Apomorphine-induced rotation was determined 10, 20, 30 days after the lesion. On post-lesion day 34, 5 rats received fetal solid SN graft (E16) in the lateral ventricle contralateral to the lesion. Rotational behavior was determined at 10, 20, 30, 45 and 60 days after the graft and from there on each 15 days for the next 4 months. Rats were sacrificed and the tyrosine hydroxylase (TH) and dopamine-B-hydroxylase (DBH) reactivity were determined. Grafted group performed less rotational behavior (RB) (down to 50%) about 145 days after the graft, whereas the sham operated group increased rotation frequency.

We propose that humoral release of neuroactive substances produces a reduction in hypersensitive dopamine-receptors, causing them to return to a more normal level following the lesion. Spiperone-Binding tests are currently underway in our laboratory to this suggestion.

353.4

RECOVERY OF MOTOR IMPAIRMENTS AND TH-IR IN AGED RATS WITH INTRAVENTRICULAR ADRENAL TRANSPLANTS. R. Drucker-Colín, F. García-Hernández*, R. Vega-Carbajo*, M. Dávila and O. Prospéro-García. Depto. de Neurociencias, Instituto de Fisiología Celular, UNAM, México.

The motor performance of aged rats suggests dopamine deficiency, since apomorphine can transiently revert motor inabilities. This suggests that deterioration of brain dopamine (DA) neurotransmission may contribute to some of the movement disorders of advanced age. This work was carried out to determine the capacity of adrenal medullary grafts to revert such motor inabilities. The effects of the grafts on nigro-striatal tyrosine hydroxylase immunoreactivity (TH-IR) was also examined. Rats of 4 and 22 months of age were tested for swimming capacity for 15 minutes. Each aged rat was then transplanted with adrenal medullary tissue into the lateral ventricle near the caudate. The effect of the graft on swimming behavior was tested 10, 20, 50 days after the transplant. The performance of grafted aged rats was significantly improved. Aged animals adopted a more horizontal position, and swimmed more vigorously and successfully. TH-IR was also improved, since the relative optical density in the nigro-striatal pathway reached levels similar to those of young rats. These data suggest that grafting adrenal medulla in aged rats may cause a shift in the relative amounts of DA. Moreover, the improvement in motor coordination suggests that adrenal implants restore age related impairments in neurotransmission.

353.5

PARTIAL REVERSAL OF POST-LESIONAL CHANGES IN THE DOPAMINE-DENERVATED STRIATUM OF THE RAT BY DOPAMINERGIC NEURONS-ENRICHED IMPLANTS.
J.P. Herman , M. Le Moal and N. Abrous (SPON: B. Dufy). INSERM U259 - Université de Bordeaux II., 33077 Bordeaux, France.

Lesion of the nigrostriatal dopaminergic (DA) pathway induces a variety of morphological and functional changes in the denervated striatum, among others DA receptor upregulation, an increase of enkephalin (ENK) synthesis and content, of neuropeptide Y (NPY) content and -following neonatal lesion- serotoninergic (5HT) sprouting. The influence of DA-enriched mesencephalic grafts on these changes were tested.

Grafts were implanted into the denervated striatum in neonatal (PD6) or adult rats, 4 days after the unilateral destruction of the nigrostriatal DA pathway. Implanted DA neurons reinnervated the host striatum and reversed the rotational assymetry evoked by amphetamine in both age group 1 month after grafting. DA receptor hypersensitivity -as judged by apomorphine-induced rotation- was equally reversed by the grafts. Changes in ENK and NPY systems could be visualized by immunohistochemistry following the lesion in both age-group; however only the increase in NPY was antagonized by the graft, while that of ENK was unifluenced. Likewise 5HT sprouting evoke by neonatal lesion was present, irrespective of the presence of the grafts. The results suggest an unequal influence of the grafts on the different target system in the striatum.

353.6

FETAL STRIATAL TISSUE GRAFTS INTO EXCITOTOXIC-LESIONED STRIATUM: PHARMACOLOGICAL AND BEHAVIORAL ASPECTS. M. Giordano, P.R. Sanberg, S.F. Calderon and A.B. Norman. Lab. of Behavioral Neuroscience, Depts. of Psychiatry, Psychology, Neurosurgery, Physiology, and Anatomy, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0559.

Although fetal striatal tissue grafts reverse the functional deficits produced by excitotoxin lesions of the striatum, relatively little is known about their pharmacological properties. We investigated drug induced behaviors and dopamine receptors in rats with fetal striatal grafts. Excitotoxic lesions of the striatum abolished haloperidol and SCH23390 induced catalepsy (1). Fetal striatal grafts (E 17-19) induced a significant increase in both haloperidol and SCH23390 induced catalepsy six weeks after the graft, but this effect was not significant at 14 or 20 weeks post transplant. Fetal striatal tissue grafted into the unilaterally lesioned striatum ameliorated apomorphine- induced rotation behavior (2). However, autoradiographic analysis of fetal grafts in both bilateral and unilateral excitotoxin-lesioned striatum demonstrated the absence of both [^{3}H]SCH23390 and [^{3}H]spiperone binding to D_1 and D_2 dopamine receptors.

In conclusion, although fetal striatal grafts are capable of normalizing spontaneous behavior (3) they have differential effects on dopamine agonist and antagonist-induced behaviors, and do not appear to express dopamine receptors.

(1) Calderon, S.F., et al., Brain Res., in press, 1988.
(2) Norman, A.B., et al., Neuropharmacol., 27:333-336, 1988.
(3) Giordano, M., et al., Brain Res. 446:183-188, 1988.

353.7

SIX MONTH NEOSTRIATAL TRANSPLANTS EXHIBIT MINIMAL CONNECTIONS WITH HOST SUBSTANTIA NIGRA. J.P. McAllister, S.R. Cober*, E.R. Schaible* and P.D. Walker. Dept. of Anatomy, Temple U. Sch. Med., Philadelphia, PA 19140.

Our previous evaluation of neostriatal grafts placed in the kainic acid lesioned neostriatum (NS) of adult rats indicated that minimal connectivity existed between the graft and the host brain at 2 months post-transplantation (Brain Res., 425: 34-44,1987). The purpose of the present study, performed at 6-8 months post-transplantation, was to determine if connectivity had changed. Five days after kainic acid lesions were made, cell suspensions of E14 neostriatal primordia were injected into the lesion site. Eight animals with grafts received unilateral injections of lectin-bound horseradish peroxidase into the ipsilateral substantia nigra. Retrograde labelling of cell bodies was conspicuous in intact regions of the host NS surrounding the graft, but no transplanted neurons were labelled. Likewise, anterograde axonal label was abundant within the intact NS and the lesion site adjacent to the transplant. However, axonal and terminal labelling was very sparse and present only in peripheral regions of the graft. Therefore, we conclude that 6-8 month old NS grafts do not make appreciable connections with the host substantia nigra. Since Pritzel et al (Exp. Brain Res. 65:112-126, 1986) have shown connections at this time with similar grafts, the possibility that differences may occur between lesioning agents will be discussed.

353.8

FETAL STRIATAL GRAFTS IN THE IBOTENATE LESIONED STRIATUM REINNERVATE THE HOST GLOBUS PALLIDUS AND RECEIVE SPECIFIC INPUTS FROM THE HOST. K. Wictorin*, O. Isacson*, R. Simerly, L.W. Swanson, C. Ouimet and A. Björklund (SPON: ENA). Dept. Med. Cell Res., Univ. of Lund, Lund, Sweden.

Graft-host connections of striatal suspension grafts in the ibotenic acid lesioned rat striatum were studied with anterograde and retrograde tracers. Injections of Phaseolus lectin into the graft labeled axons which could be traced from the graft into the host globus pallidus. Fluoro-Gold injections into the host globus pallidus labeled large numbers of graft neurons which were distributed in patches throughout the graft. Similar Fluoro Gold injections in the host substantia nigra labeled only scattered graft neurons. Immunohistochemstry of DARPP-32 (normally enriched in neurons possessing dopamine D1 receptors) revealed a patchy distribution of DARPP-32 positive neurons within the grafts, and these patches overlapped with the patches of neurons retrogradely labeled from the host globus pallidus. The DARPP-32 patches received a dense innervation of tyrosine hydroxylase positive fibers from the host substantia nigra. The grafts also received host afferents labeled by Phaseolus lectin from the frontal cortex, and these cortical afferents were seen to ramify both within and outside the DARPP-positive graft patches. The results suggest that functional graft-mediated behavioural effects may at least in part depend on a partial reconstruction of striatal circuitry.

353.9

CO-TRANSPLANTS: EFFECT OF FETAL STRIATAL TISSUE ON FETAL DOPAMINERGIC NIGRAL NEURONS. D.M. Yurek, T.J. Collier, B.F. Daley and J.R. Sladek, Jr. Department of Neurobiology & Anatomy, University of Rochester School of Medicine & Dentistry, Rochester, New York, 14642.

In the developing animal, striatal tissue provides a target for fetal nigral cells which are committed to becoming DA neurons of the nigrostiatal pathway. Several factors may contribute to the synpatic formation between these two groups of developing neurons, including guidance of axonal growth by extracellular channels, selective adhesion, and the release of chemotropic factors from the post-synaptic neurons. Menesini-Chen et al. (*Arch. Ital. Biol. 116*, 53-84, 1978) have demonstrated that intracerebral injections of nerve growth factor (NGF) can direct sympathetic fibers to project in an aberrant pathway towards the NGF injection site. This suggests that fiber outgrowth can be manipulated by the appropriate chemotropic stimuli. We examined if factors within the fetal striatal milieu serve to (1) stimulate the proliferation of fetal nigral cells with DA phenotypes, (2) if implanted proximal to the fetal nigral tissue, act as a chemotropic target and attract fiber outgrowth from fetal substantia nigra tissue grafts. Recovery of DA function was pharmacologically assessed using the rotational model.

Donor fetal striatal and nigral tissue was obtained from embryos [E15-17] of pregnant female Sprague-Dawley rats. Fetal striatal tissue was dissociated into cell suspensions. Male Sprague-Dawley rats [225-250 gm] received unilateral 6-OHDA nigral lesions, screened for amphetamine-induced rotation, and served as co-graft recepients. Fetal striatal cell suspensions were implanted 1 mm above and below the fetal nigral tissue chunk within the lateral ventricle adjacent to the DA-denervated striatum.

Tyrosine hydroxylase immunocytochemical analysis of brain slices containing the co-grafts revealed that TH-positive grafted neurons proliferated both within the nigral graft and also in the adjacent regions in which the striatal cell suspensions were located. TH-positive cells located in the region of the striatal cell suspension were robust with extensive arborization and extended processes. Moreover, some TH-positive fibers extended processes through the ependymal wall of the ventricle and innervated the adjacent DA-denervated striatum.

Pharmacological tests revealed that the co-grafts attenuated apomorphine- and amphetamine-induced rotation. Several of the co-grafted animals treated with amphetamine rotated exclusively toward the contralateral side throughout the 75 minutes of testing indicating a predominance of DA activity on the grafted side. These data indicate that fetal striatal cells may provide a neurotropic influence on the proliferation of fetal nigral grafts. This research was supported by T32 MH 18260 (DMY) and AG 00847 (JRS).

353.10

LONG-TERM SURVIVAL OF GRAFTED FETAL NIGRAL DOPAMINE CELLS AND BEHAVIOR RECOVERY IN THE RAT. H.Nishino, H.Sato*, F.Furuyama*, Y.Isobe*, T.Hashitani*, M.Kumazaki*, Y.Ishida*, R.Shibata* and T.Ono. Dept. Physiol, Nagoya City Univ., Med. Sch., 467 Nagoya and Dept. Physiol., Fac. Med., Toyama Med.& Pharmaceu. Univ., 930-01 Toyama, Japan.

Cell suspension of fetal rat substantia nigra was grafted into the ipsilateral caudate nucleus of young rats with unilateral lesion in the nigrostriatal dopamine pathway, and survival of grafted cells, recovery from motor imbalance and security for long period after the graft were investigated. The results were as follows : 1) Recovery from motor imbalance started in the 2nd week and almost fully in the 4th week after the graft. 2) Once occuring, the recovery continued for more than 2 years, although the host animals sometimes suffered from mastopathy or pituitary adenoma. 3) Morphological examination revealed that grafted cells (TH-positive cells) had spiny- and mega-neurites in the 2nd week, and developed into multigonal neurons later. 4) Animals with TH-positive cells more than 300 showed full, 50 to 300, partial, and those with less than 50, poor recovery. 5) Synaptic connections between TH-positive soma/dendrites and TH-negative soma/dendrites were detected. 6) Level of dopamine, DOPAC and HVA restored 30 to 50% of those of control level (brain dialysis, HPLC method). The data suggest that grafted cells, once survived, alleviate motor imbalance safely for more than 2 years.

353.11

HUMAN FETAL NIGRAL NEURONS GRAFTED TO DOPAMINE-DENERVATED IMMUNOCOMPROMISED RATS: HISTOCHEMICAL & ELECTROCHEMICAL INVESTIGATIONS. I. Stromberg*, P. Almquist*, M. Bygdeman*, T. Finger, G. Gerhardt, A.-C. Granholm, T. Mahalik, A. Seiger, P. Stieg*, L. Olson, B. Hoffer. Karolinska Institute and Hospital, Stockholm, Sweden; Univ. of Colorado Health Sciences Ctr., Denver, CO and Univ. of Miami Medical Ctr., Miami, FL, USA.

Human fetal substantia nigra, obtained from first-trimester elective abortions, was grafted to DA-denervated striatum of adult immunocompromised (cyclosporin-treated or athymic nude rats). The DA-denervation was made by 6-OHDA-injection into the nigrostriatal pathway and the rats were tested using apomorphine-induced rotation before grafting. The transplants were implanted intraparenchymally as small solid pieces. The survival and function of the grafts were evaluated with immunohistochemistry using antibodies against tyrosine hydroxylase (TH), laminin, 5-hydroxytryptamine (5-HT) and human-specific Thy-1. All transplants were strongly Thy-1-immunofluorescent, and weaker fluorescent processes from the grafts were seen entering the host striatum. These processes were also TH-immunoreactive. Numerous TH-positive cells were seen in the transplants. In addition, 5-HT-immunoreactive cells were often seen. Laminin-positive structures, presumably blood vessels, were widely distributed in the grafts. High-speed in vivo chronoamperometric recordings demonstrated K^+-induced monoamine overflow within and adjacent to the transplants. The ratios of oxidation to reduction currents suggested both DA and 5-HT were released. Ongoing studies will evaluate the sensitivity of the grafted human DA neurons to the neurotoxic actions of MPTP.

353.12

HUMAN XENOGRAFTS TO THE STRIATUM OF DOPAMINE-DEPLETED RATS: LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY. Thomas J. Mahalik, Ingrid Stromberg, Thomas E. Finger, Lars. Olson, Ake. Seiger, Marc Bygdeman, and Barry J. Hoffer. U. of Colorado Medical School, Karolinska Institute, U. of Miami.

Grafts of human fetal dopaminergic neurons have been suggested as a therapy for Parkinson's disease. The purpose of our study was to use ultrastructural immunocytochemistry to characterize grafts of human fetal tissue to the striata athymic rats

Donor tissue was obtained from fragments of dead fetuses derived from elective clinical abortions during the 6 to 12th weeks of pregnancy. The tissue was placed into the lesioned striata of 7 athymic "nude" rats which were allowed to survive from 3 to 5 months before being perfused.

The grafts contained tyrosine hydroxylase-like immunoreactive (THLI) and serotonin-like (5-HTLI) immunoreactive cell bodies and fibers. THLI terminals formed symmetric and asymmetric synapses, and 5HT-LI terminals formed asymmetric synapses in the host striatum. The host neuropil also contained THLI and 5-HTLI dendrites.

Our study indicates that xenografts of human fetal material can form synapses within the catecholamine-depleted rat striatum and that the grafts may be regulated by input from the host CNS.

353.13

MESENCEPHALIC TRANSPLANTS RESTORE DOPAMINERGIC MODULATION OF STRIATAL ACETYLCHOLINE AFTER 6-HDA. R.K. Carder, D. Jackson, H.J. Morris*, R.D. Lund, M.J. Zigmond. Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA. 15261

Ventral mesencephalic grafts placed into the striatum of rats depleted of dopamine (DA) as neonates, survive and send out extensive processes. These grafts appear to exert some degree of functional control over motor behavior in that these animals make contralateral rotations in response to amphetamine and stress. Biochemical examination of depleted neonates reveals a loss of inhibitory influence of endogenous DA on the release of acetylcholine (ACh) from striatal interneurons. Using an in vitro superfusion system, we sought to determine whether there was endogenous DA release from grafts, as well as what impact this DA has on ACh release.

Striatal slices were prepared from animals that had received unilateral grafts and turned at least 5 turns/min in response to amphetamine and stress. Striatal DA levels were approximately 6% of control levels as compared to 2% on the untransplanted side. Field depolarization (18 mA, 8 Hz) increased basal efflux of DA on the transplanted side as measured by HPLC while producing no change on the untransplanted side. To assess the effect of DA on ACh release, slices were preincubated with [^{3}H]choline and the efflux of tritium into the superfusate was measured in the presence or absence of the DA antagonist, sulpiride (1 um). In slices from transplanted rats, sulpride produced a 50-270% increase in ACh. In comparison, sulpiride produced no increase in ACh release from slices prepared from DA-depleted neonates and a 90% increase from normal slices. These results suggest that mesencephalic grafts are capable of releasing DA and that this released DA modulates striatal cholinergic neurons. (NIH grants EY05283 and NS19608)

353.14

EXOGENOUS D2 AGONISTS BUT NOT ENDOGENOUS DOPAMINE CONTROL ACETYLCHOLINE RELEASE IN STRIATO-STRIATAL GRAFTS. T. Wichmann*, K. Wictorin*[a], A. Björklund*[a] and K. Starke* (SPON: G. Gmelin). Inst. Pharmacol., Univ. of D-7800 Freiburg and [a]Dept. Med. Cell Res., Univ. of S-22362 Lund

Striato-striatal grafts of embryonic tissue in rats with striatal ibotenic acid lesions have dopamine D2 binding sites (Isacson et al., Neurosci. 22:481, 1987) and are invaded by host dopaminergic fibres (Clarke et al., Neurosci. 24:791, 1988). We investigated the dopaminergic control of acetylcholine (ACh) release in graft tissue 16 to 20 weeks post-transplantation.

In superfusion experiments after preincubation of tissue slices with 3H-choline the overflow of tritium upon electrical stimulation (3 Hz, 2 min), reflecting ACh release, was inhibited by the D2 agonist quinpirole (0.03 µmol/l). The inhibition was less in grafts (26%) than in controls (52%). The D2 antagonist sulpiride (1 µmol/l) enhanced ACh release in controls by 30% and after addition of the dopamine uptake inhibitor nomifensine (1 µmol/l) by 673%. Nomifensine alone and the dopamine releasing drug amphetamine (1 µmol/l) decreased ACh release in controls by 39% and 81%. However, none of these drugs had any significant effect on ACh release in grafts.

We conclude that striato-striatal grafts partly restore the impaired choline uptake in lesioned striata. Although the grafted cholinergic cells are sensitive to D2 agonists, modulation by endogeneous dopamine is almost absent.

353.15

RECOVERY FROM UNILATERAL NIGROSTRIATAL DOPAMINE DEPLETION INDUCED BY DOPAMINE-SECRETING POLYMER IMPLANTS A. Gonzalez*, P. Barton*, J. B. Becker, T. E. Robinson, A. Sintov* and R. J. Levy* (sponsor: D. Green). Dept. Psychology, Div. Pediatric Cardiology and Neuroscience Program, The University of Michigan, Ann Arbor, MI.

QUESTION: Can an intrastriatal implant of a dopamine (DA) secreting polymer matrix alleviate the effects of unilateral DA depletion (i.e., mimic the effects of tissue grafts)? In a first attempt to address this question we studied the effects of implants (6.9±0.5 mg) of a silicone polymer matrix that released DA at a slow constant rate (43.3±3.9 nmol/mg/day) on apomorphine (APO)-induced rotational behavior in male rats with a unilateral 6-OHDA lesion of the substantia nigra.

Animals that received DA-secreting implants in the medial portion of the caudate nucleus, on the denervated side, showed a 50% reduction in APO-induced rotational behavior (relative to pre-implant). This effect was significantly different from the effect of control implants and persisted unabated for 2 months (when the experiment was terminated). This reduction in rotational behavior is comparable to that seen with adrenal medulla grafts. Additional studies in progress will be reported. This study suggests that the controlled release of DA from an intracerbral polymer matrix can produce a long-lasting reduction in some of the symptoms associated with nigrostriatal DA depletion.

(Supported by NS22157 (JBB), DA04294 (TER) & HL38118 (RJL)).

353.16

Adrenal medulla grafts enhance functional activity of the striatal dopamine system following substantia nigra lesions. Jill B. Becker, William J. Freed and Eileen Curran*. Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI and The National Institute of Mental Health, St. Elizabeth's Hospital, Washington, D.C.

Adrenal medulla grafts in the lateral ventricle reduce the behavioral manifestations of striatal dopamine (DA) depletion in an animal model of Parkinson's Disease. Using microdialysis in freely moving rats, the behavioral effectiveness of adrenal medulla grafts was found to be associated with changes in neurotransmitter activity. Contrary to a popular hypothesis, baseline extracellular concentrations of DA in striatum were not elevated and DA was not detectable in cerebrospinal fluid (CSF). However, adrenal medulla grafts were associated with an increase in DA turnover and amphetamine-stimulated striatal DA release was increased in animals with behaviorally effective adrenal medulla grafts. Therefore, adrenal medulla grafts increase presynaptic striatal DA release without an appeciable increase in CSF DA. Whether this striatal DA release is under feedback control is currently under investigation.

Adrenal medulla grafts also increased serum DA concentrations, and the increase in serum DA was directly correlated with the behavioral efficacy of the grafts. We conclude that DA, produced by adrenal medulla grafts, gains access to the striatum via the blood supply and then leaks out into the host striatum through permeable blood vessels adjacent to the graft. Through this mechanism, adrenal medulla grafts may increase functional dopaminergic activity in the striatum. These results may be important for understanding the mechanism through which autografts of adrenal medulla cells produce a putative alleviation of the symptoms of Parkinson's Disease. (Supported by NIH grants #NS22157 and NS01056 to JBB).

LIMBIC SYSTEM I

354.1

ELECTROPHYSIOLOGICAL AND ANATOMICAL DEVELOPMENT OF HUMAN HIPPOCAMPUS AS INTRAOCULAR GRAFTS IN RAT HOSTS. R. Freedman, A.-C. Granholm, B.J. Hoffer, I. Strömberg*, A. Seiger, P. Stieg, M. Eriksdotter-Nilsson*, M. Byqdeman*, L. Olson. Denver VAMC, Univ. of Miami, Karolinska Institute, and Univ. of Colorado, Denver, CO 80262.

Brain fragments derived from dead first trimester abortuses were transplanted into the anterior chamber of the eye of athymic nude rats. Transplants of hippocampus were allowed to develop in the eye for up to one year. Neuroanatomical studies showed the presence of neuronal cell bodies, which in some cases formed a layer parallel to the transplant surface. Immunocytochemical evidence for intrinsic GABA-ergic terminals, as well as cholinergic and adrenergic terminals from the iris, was obtained. Neuronal action potentials were recorded extracellularly, and laminated field potentials were seen after surface stimulation. Paired pulse inhibition was also demonstrated. Seizure-like activity was seen after treatment with penicillin, enkephalin, or tetanic stimulation. The data suggest that human hippocampal transplants develop many neurobiological features which are characteristic of hippocampus in situ, including excitatory and inhibitory neuronal circuitry.

354.2

ELECTRICAL PROPERTIES AND AMINO ACID SENSITIVITY OF IDENTIFIED POSTNATAL HIPPOCAMPAL NEURONS IN CULTURE. C.F. Zorumski, L.L. Thio, and D.B. Clifford. Depts of Psychiatry and Neurology, Washington U. Sch. Med., St. Louis, MO

To study the development of electrical properties and amino acid sensitivity of postnatal hippocampal projection neurons we have used primary cultures from neonatal albino rats injected in the fornix with latex rhodamine microspheres. In 2-10 day old animals, injections label neurons in the pyramidal layer of areas CA3 and CA1 but not the dentate gyrus. Label is greater in CA3 than CA1 and labelled neurons stain with antibodies against neuron specific enolase but not GFAP.

Over the first 10 days in culture , input resistances fall from >2000MΩ to near 450MΩ while capacitance increases from 25pf to >70pf. Membrane potentials remain stable over this time and all neurons fire action potentials (AP). AP amplitudes remain greater than 70mV but AP durations decrease from 14ms to 5ms. Membrane charging curves are well described by a monoexponential process for 87% of neurons studied within 5 days of plating. By day 10 only 39% have monoexponential curves reflecting increases in cell size and neuritic processes.

Neurons respond to all excitatory amino acids and GABA. Responses decline over the first 24 hours in culture and recover over several days. Responses to glutamate at all ages consist of two components, a rapidly activating and desensitizing phase followed by a slower decaying tail.

354.3

DENTATE GRANULE NEURONS FROM TWO POPULATIONS OF SPRAGUE-DAWLEY RATS HAVE DIFFERENT BRANCHING PATTERNS, BUT SIMILAR DENDRITIC LENGTHS. B.J. Claiborne, M. Lehman* and L. Rihn*. Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX 78285.

The dendritic branching patterns of dentate granule neurons in two populations of adult Sprague-Dawley rats were compared. Dorsal blade granule neurons (n=12) in rats obtained from Harlan Sprague-Dawley Inc. were filled with horseradish peroxidase, analyzed in three dimensions and compared to granule neurons (n=30) in Sprague-Dawley rats from Zivic Miller described previously (Claiborne et al., *J. Comp. Neurol.*, in press). Results showed that the molecular layer was significantly wider in the Harlan rats (299±11 um vs. 257±7 um; M±SEM), but that neurons from the Harlan population had fewer dendritic segments (27 ± 1) and smaller transverse spreads (318 ± 24 um) than did neurons from the Zivic Miller sample (31 ± 1 and 347±14 um). Because segment number and transverse spread varied inversely with layer width, total dendritic lengths per neuron were similar in the two populations. Two representative Harlan neurons had lengths of 3148 and 3543 um; the mean dendritic length of Zivic cells was 3478±88 um. These findings suggest that neurons may vary dendritic parameters systematically in order to conserve total dendritic length.

(We thank H.W. Nevin for the computer software. Supported by NIH grant AG 07141)

354.4

LONG LASTING INDUCTION OF PERIODIC SYNAPTIC CURRENTS BY NMDA IN IMMATURE HIPPOCAMPAL NEURONS IN ABSENCE OF SYNAPTIC TRANSMISSION.
E. Cherubini, K. Krnjevic and Y. Ben-Ari, INSERM U-29, 123 Bd Port-Royal, 75014 Paris, FRANCE.

The single electrode voltage clamp technique was used to study the action of NMDA in CA3 immature rat hippocampal neurons (1-10 days) in the in vitro slice preparation. Slices were perfused with ACSF containing TTX (1 uM) to block fast Na^+ currents and Cs^+ (4 mM) and TEA (10 mM) or 4-AP (30 µM) to reduce outward K^+ currents. Bath application of NMDA (5-10 µM) induced a slow inward current which was blocked by APV (50 µM). In addition, after repeated (3-5) applications of NMDA, Periodic Inward Currents (PICs) developed, which were maintained for several hours after NMDA was washed out. NMDA was essential for their induction but not for their maintenance, since APV failed to block them, once they have developed. The amplitude of PICs was a monotonic function of the holding potential and their reversal was close to 0 mV. PICs were blocked by a Ca^{2+} free medium or by Ca^2 antagonists Cd^{2+} (50 µM) or Co^{2+} (2 mM). PICs were recorded with both K Cl or K-acetate containing microelectrodes, as well as with electrodes containing the calcium chelators EGTA or BAPTA. PICs were not blocked when TEA (126 mM) was substituted for external Na^+. PICs were reduced in frequency by bicuculline (10-20 µM) and completely abolished by kynurenic acid (1 mM).

We conclude that PICs represent a novel mechanism of long lasting synchronization of synaptic currents by NMDA in absence of synaptic transmission.

354.5

ONTOGENY OF EXCITATORY PROCESSES IN THE RAT CA1 HIPPOCAMPAL REGION. E.W. Lothman and H.B. Michelson. Dept. of Neurology, Univ. of Virginia Medical Center, Charlottesville, VA 22908.

Although several studies have compared hippocampal slices from young versus adult rats, a systematic in vivo characterization of the ontogeny of electrophysiologic responses in this structure has not yet been done. This and the accompanying study report responses obtained from rats of postnatal (PN) ages 7-65 days. Under urethane anesthesia, a stimulating electrode was placed in one CA3 region and a second recording electrode in the contralateral CA1 region. Stimulus intensity was then varied to generate input-output curves for CA1 population spikes (PS). In younger animals, PS were broader, smaller in amplitude, and required higher intensity stimuli. However, after PN 14, excitability (measured by voltage required for half maximal PS) and PS duration (width at one-half peak for maximum amplitude) were not different from adult values. In contrast, maximum amplitude PS did not attain adult levels until PN 35. Conduction velocities (measured from time of stimulation to peak of evoked PS) steadily increased from PN 7 to PN 65. Thus, many, but not all, aspects of excitatory responses in CA1 hippocampal region are mature by PN 14.

354.6

ONTOGENY OF PAIRED PULSE INHIBITION IN THE RAT CA1 HIPPOCAMPAL REGION. H.B. Michelson and E.W. Lothman. Dept. of Neurology, Univ. of Virginia Medical Center, Charlottesville, VA 22908.

To complement our studies on the ontogeny of excitatory processes in the hippocampus, in vivo studies on the maturation of inhibition were carried out. Paired pulse stimuli were given in CA3 and population spikes (PS) recorded in the contralateral CA1 of rats on postnatal (PN) day 7 to PN 65. Ratios of the second (test) PS [PS(T)] amplitude to that of the first (conditioning) PS [PS(C)] assessed the potency of inhibition. We systemically explored the influence of PS(C) and interpulse interval (IPI) on paired pulse inhibition. Before PN 14, no inhibition was present at any amplitude PS(C) or any IPI. At later time points, inhibition appeared for IPI of 20-100 msec, provided PS(C) amplitudes were >75% of maximum. Inhibition was first detected at PN 18 and gradually increased to adult levels between PN 42-65. These studies indicate that recurrent GABAergic inhibition in the CA1 area of the hippocampus is not functionally present for at least 2 postnatal weeks and delayed for as much as 2 months before reaching adult values. The time course for this development lags considerably behind that of "excitatory" processes outlined in the accompanying abstract.

354.7

DEVELOPMENT OF PAIRED-PULSE SYNAPTIC FACILITATION IN THE RAT HIPPOCAMPUS, AREA CA1. P.G. DiScenna & T.J. Teyler Neurobiology Department, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

We are examining the development of one measure of neuronal excitability, paired-pulse synaptic facilitation, in the rodent hippocampus, area CA1. Hippocampal slices are prepared from 2-4,6,8,10,13,15,20 and >60 day rats, and maintained using standard slice procedures (2mM Ca/2mM Mg). At least 4 animals and 8 slices have been sampled at each age. Stimulating (50um concentric bipolar) and recording electrodes (2-5mOhm) are placed, on-line, in the middle of the apical dendritic field of CA1, in order to record the evoked synaptic field potential. Homosynaptic paired pulses (conditioning pulse = 40-60% max. field EPSP) are recorded across a range of interpulse intervals (10-5000ms), delivered at .033Hz. We are comparing the initial slopes of the conditioning and test pulse field EPSPs, as well as longer latency events, both within and across ages. Preliminary analyses demonstrate the presence of PPF at the earliest day tested, PN2 (eg. 2.58% @ 20ms IPI; 16.98% @ 60ms; 12.98% @ 120ms; -9.97% @ 500ms), with gradual increases peaking in the 15 day slices (120% @ 20ms; 134.58% @ 60ms; 86.73% @ 120ms; 25.48% @ 500ms), with a slight decline in the >60 day group (87.8% @ 20ms; 96.33% @ 60ms; 63.25% @ 120ms; 18.22% @ 500ms). Excitatory processes appear to develop before inhibition, in CA1. Therefore the decreased level of PPF during the early postnatal days, provides a margin of safety for the developing hippocampus. Supported by EPA, NIH, ONR.

354.8

SPROUTING OF CENTRAL AND PERIPHERAL NORADRENERGIC FIBERS INTO THE DENTATE GYRUS FOLLOWING COMBINED LESIONS OF THE ENTORHINAL AND SEPTAL AFFERENTS. G.M. Peterson, Dept. Anat. Cell Biol., East Carolina Univ. Sch. of Med., Greenville, NC 27858.

Virtually all of the afferents to the hippocampal formation (HF) undergo reactive synaptogenesis (sprouting) after removal of adjacent afferent systems. However, the noradrenergic (NA) afferents, which in other regions of the brain demonstrate a remarkable propensity for regeneration and sprouting, do not sprout in the denervated HF. In contrast, sympathetic NA axons, which do not normally invade the CNS, sprout into the HF following interruption of the septohippocampal cholinergic input. The present study was designed to determine if interruption of both entorhinal and septal inputs can alter the innervation pattern of central or peripheral NA afferents to the dentate gyrus (DG) of adult female Sprague-Dawley rats. The ipsilateral and contralateral entorhinal afferents to the right DG were removed by knife cuts through the ipsilateral perforant path and the ventral hippocampal commissure, respectively. At the same time, or 4 weeks later, the fimbria-fornix was bilaterally aspirated. Four weeks later the animals were perfused, the brains removed, and 40 um thick sections were cut in either the coronal or horizontal plane. Lesions were verified in sections stained for Nissl, Timm's silver sulfide, or acetylcholinesterase histochemistry. The noradrenergic identity of the fibers was indicated by immunoreactivity for dopamine beta hydroxylase (DBH) and peripheral sympathetic fibers were demonstrated by immunoreactivity for nerve growth factor receptor (NGFr). DBH + fibers were present in the hilus of both the left and right DG and many of these were also found to be NGFr +. The outer half of the molecular layer of the right DG had a dense plexus of DBH + fibers coursing parallel to the pial surface. However, no NGFr + fibers were present in this region, thus indicating that the DBH + fibers are of central origin. It is concluded that combined bilateral deafferentation of the entorhinal and septal inputs does not change the sprouting patterns of peripheral sympathetic fibers but results in the sprouting of central NA fibers in the molecular layer of the dentate gyrus. (Supported by NC United Way and the Alzheimer's Disease and Related Disorders Association.)

354.9

PEPTIDERGIC NEURONS IN THE HUMAN HIPPOCAMPUS DURING DEVELOPMENT AND AGING. F. Lotstra* and J.-J. Vanderhaeghen. Laboratory of Neuropathology and Neuropeptide Research. Brugmann and Erasme Hospitals, Université Libre de Bruxelles, Brussels, B-1070, Belgium.

The distribution of Neuropeptide Y (NPY), Neurotensin (NT) Somatostatin (SOM), Cholecystokinin (CCK), and Substance P (SP) neurons is studied in the human hippocampal formation using the peroxidase technique of Sternberger. CCK and SP interneurons are mainly represented in the hilus, CA3 and CA2. NPY and SOM are more concentrated in CA1, subiculum and entorhinal cortex. Several interstitial NPY, SP and SOM neurons are present in the angular bundle. Before three years of age, transient immunoreactivity is present in some neurons. Such transient neurons are CCK interneurons in the entorhinal cortex, NT pyramidal neurons in the subiculum and NT granular neurons in the area dentata. Althought the density of NPY neurons decreases during development, the total number of these neurons is well established at birth, stays stable until at least the age of 42 and begins to decrease at the age of 60. Similarly the total number of SP neurons in the hilus region stays stable from birth until the age of 42. Supported by Belgian FRSM 3.4523.86 and FMRE 86.

354.10

LOCALIZATION OF SYNAPTIC VESICLE PROTEIN IN DEVELOPING RAT PIRIFORM CORTEX. L.E. Westrum, R.E. Westenbroek*, N.A. Nousek-Goebl*, and W.D. Matthew[1]. Depts. of Neurol. Surg., Biol. Struct., and Pharmacol., Univ. of Washington, Seattle, WA 98195; [1]Dept. of Neurobiol., Harvard Med. Sch., Boston, MA 02115.

Antibodies to synaptic vesicle protein (J. Cell Biol. 91:257, 1981) are being used to study synaptogenesis and plasticity in piriform cortex (PC) using immuno-labeling techniques. A banding pattern which delineates sublaminae of PC is lost following olfactory bulb lesions. The ultrastructural basis for these patterns is being examined in rats of postnatal (PN) ages 3, 7, and 13 days and adults. Axon terminals, some with synaptic contact, and their preterminal axons label in the youngest animals. Greater numbers of terminals and synapses label in older ages whereas preterminal axons disappear after PN 7. In some of the adult material essentially all of the terminals label. The deeper sublamina (Ib) has more labeled terminals than superficial regions (Ia) with a transition zone. Label occurs in association with the surface of synaptic vesicles that are usually round in shape, and the contacts are mostly asymmetric. Postsynaptic elements do not label. The results explain the banding pattern, identify the precise synaptic elements and substructural localization and provide a valuable method for studying plasticity at a synaptic level. (Supported by NIH Grants NS09678 and DE04942. LEW is an affiliate of the CDMRC.)

354.11

BEHAVIORAL MATURATION AND PROTRACTED POSTNATAL PROLIFERATION OF RECURRENT MOSSY FIBER COLLATERALS IN THE GUINEA PIG HIPPOCAMPUS. D.P. Wolfer* and H.-P. Lipp. Institute of Anatomy, Univ. of Zürich, CH-8057 Zürich, Switzerland.

Behavioral maturation may depend on a protracted differentiation of neuronal circuitry long after the differentiation of sensori-motor systems (Flechsig's rule). The evidence for this rule is based on myelination cycles and protracted neurogenesis of hippocampal granule cells, but clear changes in the distribution of axonal terminals have not been observed thus far.

Fifty-six guinea pigs, precociously born animals, were studied (8 per age group) at the age of 5, 10, 20, 40, 80, 160, and 320 days. Behavioral tests measured activity in a novel environment, and responses to electrical shock in a shuttle-box. The area of a plexus formed by recurrent mossy fiber collaterals below and above the granule cell layer of the fascia dentata was assessed by means of digital image analysis. These mossy fiber collaterals proliferate till the age of 40 days, and thereafter in some but not all animals. The prepubertal proliferation (which may reflect mossy synapses overgrowing inhibitory interneurons) was coincident with an increase of shock levels required to induce locomotion. No age-related differences were found for the latency to start locomotion in a novel environment. So, Flechsig's rule applies to mossy fibers, too, and their late proliferation may have specific behavioral consequences. Supp. by SNF 3.081-0.84.

354.12

ISOLATION "STRESS": REARING EFFECTS UPON ACTIVITY, PLASMA GLUCOCORTICOIDS AND BRAIN GLUCOCORTICOID BINDING. R. Holson, P. Sullivan*, A. Scallet, S.F. Ali, and B. Turner*. Div. Reprod. & Develop. Toxicol., National Center for Toxicological Research, Jefferson, AR 72079 and *Quillen-Dishner College of Medicine, Johnson City, TN 37614.

It has been suggested that isolation rearing is a "stressor". To investigate this contention, male and female rats were reared from weaning at postnatal day (PND) 21 to adulthood (PND 100) in one of three standard laboratory conditions: three animals to a transparent acrylic cage (SOC), isolation in the same plastic cage (IPC) or isolation in smaller metal hanging cages (IHC). When tested in the open field, SOC activity was much greater if odor cues from cagemates were present in the field. IHC rats displayed profound freezing, due to lack of familiarity with the human handling provided by twice-weekly cage changes of plastic cages. IPC rats were more active than SOC rats without but not with cagemate odor cues in the field. There was no rearing effect on basal plasma glucocorticoids, on hippocampal or prefrontal type I or type II glucocorticoid receptors, or on body-weight-adjusted adrenal and thymus weights. Corticosterone levels following open field were only elevated in IHC. These data provide scant support for the position that isolation rearing is a chronic stressor. They do demonstrate that isolation rearing effects result from a complex interaction between prior experience and test circumstances.

357

NEW GENES FROM OLD DISEASES. X.O. Breakefield[1], F.S Collins[2], J.F. Gusella[3], R.G. Worton[4] and R.A. Weinberg[5]. [1]E.K. Shriver Ctr. and Massachusetts Gen. Hosp., Boston, MA; [2]Univ. Michigan Med. Sch., Ann Arbor, MI; [3]Harvard Med. Sch. and Massachusetts Gen. Hosp., Boston, MA; [4]Hosp. Sick Children, Toronto, Canada; [5]Whitehead Inst., M.I.T., Cambridge, MA.

Molecular genetic studies have elucidated the chromosomal location of a number of genes causing inherited neurologic and psychiatric diseases. For several of these diseases, new genes important in the function of the nervous system have been identified. This symposium will focus on genetic strategies used to find and characterize disease genes and their products. Basic recombinant DNA techniques used to proceed from linked markers to disease genes will be explained: "jumping" libraries, pulsed field gel electrophoresis, cloning in artificial yeast chromosomes, subtractive DNA libraries, and polymerase chain reaction amplification and sequencing. Xandra Breakefield will present an overview of current progress in identifying genes associated with diseases of the nervous system. Progress toward finding the genes responsible for neurofibromatosis (NF1) and Huntington disease will be presented by Francis Collins and James Gusella, respectively. Ronald Worton will discuss the structure of the X-linked gene responsible for Duchenne muscular dystrophy, and its encoded muscle protein which has homologies to alpha-actin. Robert Weinberg will describe the recessive oncogene responsible for retinoblastoma tumors and the possible role of the encoded protein in regulating gene expression in retinal cells. This symposium will alert neuroscientists to new genes important in the nervous system which need further characterization.

358

WORKSHOP. NON-UNIFORMITY OF SYNAPTIC PHYSIOLOGY: IMPLICATIONS FOR PLASTICITY IN THE NERVOUS SYSTEM. J.P. Tremblay, Laval Univ. (chairman); H. Korn, Inst. Pasteur, Inserm, Paris; A.D. Grinnell, UCLA Sch.Méd.; J.P. Tremblay and R. Robitaille, Laval Univ.; M. Wojtowicz and A. Atwood, Univ. Toronto; E. Henneman, Harvard Med. Sch.; S. Redman, John Curtin Sch. Med.

During recent years, several laboratories have presented evidence that all the synaptic contacts made by terminals of a given axon do not participate equally in the release of neurotransmitter. On the other hand, many analyses of morphological changes in central synapses have been based on the assumption that morphologically defined synapses are physiologically meaningful. Non-uniformity of synaptic physiology implies that many morphologically defined synapses are normally impotent, but may become active during adaptive or developmental changes in the nervous system. It is the purpose of this symposium to present and discuss various mechanisms that have been advanced to account for the heterogeneous behaviour of synapses; some deal with axonal properties, others raise questions about the probability of exocytosis at a given site following a propagated impulse. Korn will present evidence that synaptic noise can be reconstructed using experimental data indicating that release is quantal and well described by a simple binomial process at the level of each presynaptic interneuron. Synaptic noise than appears to be equivalent to temperature in models of neural networks. Grinnell will show that even in a relatively simple synapse (the frog NMJ), different transmitter release sites show a gradient in their participation in evoked release. He will review possible morphological and physiological explanations for these gradients. The results of Tremblay and Robitaille indicate that there are also gradients during spontaneous release and that the MEPP amplitudes produced by the distal portion of the nerve terminal are smaller. Wojtowicz and Atwood will present evidence that in the crayfish NMJ, there are variations in the number of physiologically participating synapses (n) during presynaptic inhibition and long term facilitation. These results suggest that the large number of release sites may be the substrate for such plasticity. Henneman's results suggest that impulses conducted in a single Ia afferent fiber do not necessarily activate all the synapses formed by that fiber on a motoneuron. Redman will review the evidence that different excitatory synapses arising from the same axon and contacting the same neuron can have different release probabilities, and that these probabilities are altered during PTP and presynaptic inhibition.

CELL LINEAGE AND DETERMINATION II

359.1

DIVISION PATTERN OF THE PRECURSER CELLS TO THE PERIPHERAL NERVOUS SYSTEM IN WILDTYPE AND MUTANT DROSOPHILA EMBRYOS. R. Bodmer, R. Carretto & Y.N. Jan. SPON: K. Ocorr. Howard Hughes Med. Inst., UCSF, San Francisco, CA 94143-0724.

The division patterns of the cells that give rise to the embryonic peripheral nervous system (PNS) were studied using the thymidine analog BrdU to monitor DNA synthesis (Gratzner, H.G., Science, 218: 474, 1982). It was found that cell divisions during neurogenesis of the PNS happen between ca. 6 and 9 h after fertilization. During this period, the cell divisions that occur in the epidermal cell layer of the body wall almost exclusively give rise to the PNS. Furthermore, sensory organs of different types and locations within a segment have a stereotyped pattern and time of birth. For example, the precurser cells for the external sensory (es) organs are the last ones to complete their cell divisions.

This method provides a very useful means of analyzing mutations that affect PNS development. For example: in embryos that are homozygous for mutations in the achaete-scute complex (AS-C), the PNS is reduced in that all es organs and certain other sensory neurons are absent (Dambly-Chaudiere, Ch. and Ghysen, A., Genes & Dev., 1: 297, 1987). These mutations could either be affecting the relevant precursor cells or they could disrupt the normal differentiation of their progeny. By labelling mutant embryos with BrdU it was possible to distinguish between these possibilities by showing that the precursor cells for the missing sensory organs are either absent or fail to divide. This methodology is currently being used to study other mutants that may affect the development of the embryonic PNS of Drosophila.

359.2

LOCALIZED EXPRESSION OF THE CHICKEN ENGRAILED GENE IN EARLY CHICK EMBRYOS. C.A. Gardner*#, D.Darnell*@, C.P.Ordahl*@, K.F.Barald# (SPON: M. Alpern) Dept. Anatomy & Cell Biol., U. Michigan#, Ann Arbor, MI 48109; Dept. Anatomy, U Cal San Francisco@, CA, 94143.

Engrailed (en) has been identified in Drosophila as an important developmental gene involved in the control of segmentation. A chicken gene (ChickEn) containing a region homologous to the en homeobox and to 22 additional base pairs of the fly gene has been identified [DD,CO]. A 500 bp fragment of ChickEn genomic DNA was used to make ^{35}S-labeled RNA probe [CG,KB]. In Northern analyses, the probe hybridized to 3 bands of whole cell RNA from 2 or 4 day chick embryo (5.0, 3.5, 2.2 Kb) [DD,CO,CG,KB]. In situ hybridization [CG,KB] to 4 day chick embryos (ED-4) shows heavily labeled cells in the mesencephalon. In cultured chick cranial neural crest (NC) cells (31 hr; stage 9) in situ hybridization [CG,KB] shows probe localization in a subset (<10%) of NC cells. Inv-49 is a monoclonal antibody (MCA) which Patel, Coleman, Poole, Kornberg and Goodman (ms in prep.) have shown binds to the homeo box region of the en protein product. Patel et al and we have shown that this MCA labels mesencephalon and anterior rhombencephalon of stage 9-26 chick. The MCA also stains sections of ED-4 chick in the same region of the mesencephalon in which ChickEn mRNA is localized [CG,KB]. We have found no MCA staining in chick embryos before stage 9. We are presently examining NC cells and early embryos with a variety of other antibodies for NC including CG-1 and CG-4 that label a subset of mesencephalic NC cells [KB] to determine ChickEn mRNA and MCA colocalization patterns.

359.3

NEURONAL REGULATION OF GLIAL PROGENITOR CELL DIFFERENTIATION. J.M.Levine, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook,N.Y.,11794

Astrocytes and oligodendrocytes develop from a common precursor cell termed an O2A progenitor cell. Progenitor cells have been studied extensively in cultures of post-natal rat optic nerve which contain no neurons. We have identified cells in cultures of post-natal rat cerebellum (CBL) whose antigenic phenotype is identical to that of O2A progenitor cells. However, these CBL cells rarely develop into oligodendrocytes when grown in a chemically defined,serum-free medium.

These observations suggest that neurons may influence the choices made by developing progenitor cells. This hypothesis was tested by growing post-natal day 5-7 rat optic nerve cells in serum-free medium that was conditioned by either CBL interneurons (NCM), CBL astrocytes (GCM) or several neuronal and glial cell lines. The numbers of progenitor cells and oligodendrocytes was determined by immunofluorescence staining with cell-type specific marker antibodies 18-24hr after plating the cells and again 3-5 days later. The newly plated cells contained approximately equal numbers of progenitor cells and oligodendrocytes. After 3 additional days in unconditioned defined medium, most of the progenitor cells disappeared and 85% of the labeled cells were oligodendrocytes.In contrast, after 3 days in NCM, 60% of the labeled cells were progenitor cells and 38% were oligodendrocytes. Between 3-8% of the progenitor cells were dividing in NCM suggesting that differentiation was not delayed due to extensive cell division. Medium conditioned by glial cells had no effect on the normal differentiation of oligodendrocytes.

These results indicate that CBL interneurons secrete or shed factors into the medium that effect glial development. These factors can promote the survival of progenitor cells and prevent the development of oligodendrocytes. Thus the neuronal microenvironment may play a role in regulating the differentiation of multipotential glial precursor cells.

359.4

A FINELY VARIEGATED, HOMOGENOUS MIX OF NEURON GENOTYPES EXISTS THROUGHOUT THE CNS OF MOUSE CHIMERAS. A. Peterson1,J.P. Julien*2,I.Tretjakoff*1,P.Valera*1 and O. Mayor*1. 1 Ludwig Institute, Montréal, 2 Institut du Cancer de Montréal, Canada.

Transgenic mice containing the human neurofilament light gene (hNF-L) were used to investigate the cis sequences which confer neuron specific expression and to provide a genotype marker. The hNF-L gene is expressed in neurons, the transgene product assembles into neurofilaments and is detectable in situ with a monoclonal antibody (Julien et al., Genes. Dev. 1:1085, 1987).

To investigate the lineage/clonal relationships that are postulated to underlie the origins of phenotypically different neurons, hNF-L↔+/+ chimeras were examined for the distribution of the two neuron genotypes. Throughout the nervous system, a finely variegated pattern of mosaism exists and, in each chimera similar genotype proportions were found in all neuronal populations.

These findings require that the chimera neuroectoderm was a homogenous mix of the two genotypes and that different neuronal phenotypes do not arise clonally from small numbers of prespecified progenitors. Rather, neurons of each phenotype arise from a representative sample (i.e. a large number) of progenitors, or more likely, all progenitors contribute daughter cells to all of the neuronal subpopulations at each level of the neuroaxis.

359.5

DIVERSE NEURONAL TYPES ARISE FROM A SINGLE PROGENITOR IN CHICK OPTIC TECTUM: STUDIES USING A ROUS SARCOMA VIRUS AS A LINEAGE MARKER. G.E. Gray*[1], J. Majors*[2], and J.R. Sanes[1], Depts. of [1]Anatomy & Neurobiology, and [2]Biological Chemistry, Washington Univ. School of Medicine, St. Louis, MO 63110

We are using recombinant retroviruses to trace cell lineage in the chicken optic tectum. These replication-defective vectors insert the E. coli β-galactosidase (lacZ) gene into the genome of an infected cell; the cell's lacZ-positive progeny are detected histochemically (Sanes et al., EMBO J. 5: 3133, 1986). Previously we infected tectal progenitors during the period of active proliferation, then analyzed clones when proliferation was nearly over but before laminae formed (E7-9). We found that lineally related cells form radial arrays in the developing tectum (Glover et al., Soc. Neurosci. Abstr. 13: 183, 1987). We have now extended these studies to analyze the disposition and phenotype of clonally related cells in mature tecta. We find that some (but not all) clones remain radially organized at E19, when the tectum is fully laminated. These clones span many laminae, and individual clones contain neurons of varied (≥4) distinctive morphologies, many of which can be classified by reference to Golgi studies of Cajal and others. Thus, a single precursor can give rise to neurons of diverse types.

Our new studies benefit from a novel recombinant Rous sarcoma virus that bears the lacZ gene. The viral genes pol, env, and portions of gag and src were replaced with lacZ; the vector encodes a gag-lacZ fusion protein. The helper-free virus was grown in a cell line, made by cotransfecting a quail fibroblast line (QT6) with the gag-lacZ plasmid and a plasmid that carries viral structural genes. The resulting virus has both a higher titer and a higher level of lacZ activity per infected chicken cell than we had obtained in initial studies (op. cit.) using a Moloney murine leukemia virus.

359.6

CELL LINEAGE ANALYSIS IN THE EMBRYONIC MOUSE RETINA BY RETROVIRAL VECTOR-MEDIATED GENE TRANSFER. D. L. Turner*, E. Y. Snyder* and C. L. Cepko (SPON: D. Potter). Dept. of Genetics and Program in Neuroscience, Harvard Med. School, Boston, MA 02115.

We have previously described the use of retroviral vector-mediated gene transfer as a cell lineage marking system in the mammalian nervous system (Price et al., PNAS 84:156, 1987). The BAG vector expresses the E. coli β-galactosidase gene, which permits histochemical identification of marked clones derived from single infected progenitor cells. We have used this marking system to analyze cell lineage in the postnatal rat and mouse retinas (Turner & Cepko, Nature 328:131, 1987, and unpublished results). Labeled clones contained up to three different neuronal and/or glial cell types in various overlapping combinations. This suggested that the four cell types generated in the postnatal rodent retina (rod photoreceptors, bipolar cells, amacrine cells, and Müller glia) arise from a common progenitor.

We have now extended this analysis to the embryonic mouse retina. Using the *exo utero* surgical procedure developed by Muneoka et al. (J. Exp. Zoo. 239:289, 1986) we injected BAG virus between the retina and pigment epithelium of CD-1 mice on the 14th day of gestation (plug = day 1). Approximately 400 clones were labeled in the retinas of three animals which survived to adulthood. Clones contained from one to more than 70 cells in radial clusters. Some clones contained labeled cells of all four cell types labeled in the postnatal infections. Other clones contained rods, bipolar cells, amacrine cells, and either cone photoreceptors or ganglion cells (two cell types that only form prenatally). These results confirm the proposal that rods, bipolar cells, amacrine cells, and Müller glia can arise from a common progenitor. In addition, the same progenitor can generate cones or ganglion cells earlier in development. This suggests that lineage is probably not involved in cell type determination in the rodent retina. Supported by NEI 1 RO3 EY07331-01 and NIH RO1 NS 23021-01.

359.7

CORTICAL COLUMNS ARE NOT CLONAL BOUNDARIES. C. Walsh and C.L. Cepko. Dept. of Genetics, Harvard Med. Sch., and Dept. of Neurology, Mass. Gen. Hosp., Boston, MA.

We tested the suggestion that neurons in a cerebral cortical column share common lineage by using retroviral mediated gene transfer to mark cortical progenitors(Turner,D. and Cepko,C.L., Nature 328:131,1987) in 62 fetal rats from E14-E19. A replication-incompetent retrovirus vector carrying a histochemical marker gene, E. Coli beta-galactosidase, was injected into the lateral ventricle, and rats were killed 3-60 days later and processed for histochemistry. Infection by the defective virus stably transfers the beta-galactosidase gene to neural progenitor cells, producing groups of labelled, clonally related cells. Since the virus is replication- defective, there is no ongoing infection.

After 10-60 day survival times, labelled cells showed "Golgi like" staining, allowing easy identification of cell type in most cases. All known cell types were labelled, including neurons of diverse morphology in all cortical laminae and regions. Surprisingly, it was very rare for more than two labelled neurons to occupy the same cell column. Labelled neurons occurred in small groups after E16-17 injection, generally with 2-3 neurons in the several cortical layers, separated laterally by 25-200μm. Larger groups of labelled neurons occurred after E15 injection, with 5-10 neurons in all cortical layers, spread over a similar or larger distance across the cortex.

After 3-7 day survival times, labelled, clonally related cells were more closely grouped in the subventricular and intermediate zones, but their morphology suggested migration along several, sometimes nonadjacent, radial glial cells. Thus, the widely separated neurons seen after longer survival times appear to be clonally related. Therefore, clonally related cortical neurons migrate along different radial glial cells to achieve substantial circumferential separation in their final position. Grant support from NEI 1 RO3 EY07331-01 and NIH RO1 NS 23021-01.

359.8

A COMMON PRECURSOR FOR ASTROCYTES AND OLIGODENDROCYTES. E.F. Ryder*, C. Walsh, and C.L. Cepko. Dept. of Genetics, Harvard Med. School, and Dept. of Neurology, Mass. Gen. Hosp., Boston, MA.

Macroglia have traditionally been divided into fibrous and protoplasmic astrocytes, and perineuronal and intrafasicular oligodendrocytes. However, morphological types intermediate between these classes have also been recognized (e.g., del Rio-Hortega, Mem. de la Real Soc. Esp. de Hist. Nat.14:5, 1928). Recent in vitro work (Raff et al.,Nature 303:390,1983) suggests that fibrous astrocytes and intrafasicular oligodendrocytes share a common progenitor, while protoplasmic astrocytes derive from a separate lineage (Miller and Raff, J.Neurosci. 4:585,1984). In order to show in vivo lineage relationships directly, progenitor cells were infected with a replication defective retroviral vector carrying the beta-galactosidase gene. This gene is transmitted only to progeny of infected cells. Sixty-two rats from E14-P0 (also used in an accompanying study, Walsh,C. and Cepko,C.L., this volume) received injections into the lateral ventricle and were processed for beta-galactosidase histochemistry 3-60 days later.

Labelled cells showed intense, "Golgi-like" staining of cellular processes, which allowed identification of cell type in 80-90% of cells, following survival to P8 or longer. All four types of glia could be recognized, although some labelled glia were of intermediate morphological type. Labelled glial cells occurred in groups, presumably clonally related, of up to 180 cells. Although many smaller groups contained just one morphological cell type, other groups contained any possible combination of fibrous/protoplasmic astrocytes and perineuronal/intrafasicular oligodendrocytes. Larger groups spread over wide distances in the brain and usually contained several, sometimes all four, glial types. Thus, a common progenitor apparently produces all four major morphological types of macroglia in the rat forebrain. Grant support from NIH RO1 NS 23021-01, NIH 5 T32 GMO7196, and NEI R03 EY 07331-01.

359.9

EFFECTS OF DIBUTYRYL CYCLIC AMP ON RAT RETINAL GERMINAL NEUROEPITHELIAL CELL PROLIFERATION. T.A. Reh and M. Taylor*, Dept. Med. Physiology, Univ. Calgary, Alberta, T2N 4N1.

Little is known about the factors that regulate neuronal production during development in the mammalian CNS. The purpose of this study was to examine the role of second messenger systems, specifically the cyclic AMP (cAMP) cascade, in the regulation of cell proliferation during the pre and postnatal developmental period in the rat retina. Aggregate cultures of embryonic (E15 and E18) and postnatal (P1) retina were exposed to dibutyryl cAMP (1 mM) for 48 hours. Cell proliferation and newly synthesized protein levels in the cultures were determined by TCA precipitation following incubation with H^3-thymidine (24 hours) and S^{35}-methionine (3 hours). In cultures of E15 retina, no differences were seen between control and treated groups in cell proliferation and protein synthesis; however, in E18 and P1 cultures protein levels remained constant while cell proliferation decreased to 12-20% of the control values. Histological analysis using toluidine blue stained 4 um plastic sections confirmed that the change in H^3-thymidine incorporation in the P1 cultures was due to a decline in germinal neuroepithelial proliferation. By contrast, the dibutyryl cAMP treatment had no effect on the proliferation of germinal cells from the E15 retina. These results indicate that the regulation of germinal neuroepithelial cell proliferation involves the cAMP second messenger system after E18; however, prior to that time, the proliferation of these cells is independent of changes in cAMP.

359.10

DEVELOPMENTAL IMPLICATIONS OF THREE-DIMENSIONAL DISTRIBUTIONS OF PEPTIDERGIC PERIKARYA IN THE BRAIN OF FROGS. K.M. Conway[1] and R.T. Zoeller[1,2], [1]NINCDS and [2]NIMH, Bethesda, MD 20892.

In order to compare distributions of neurons with vasotocin (AVT), mesotocin (MT), and thyrotropin-releasing hormone (TRH) phenotypes we prepared multiple sets of semi-serial transverse sections from brains of adult Xenopus laevis and Bufo marinus frogs for in situ hybridization and immunocytochemistry. Antisense DNA oligomers were synthesized using published sequences of cDNAs for Xenopus TRH and Bufo japonicus AVT and MT. The AVT and MT probes were positive in Bufo marinus, and the TRH and MT probes were positive in Xenopus. Antibody reagants specific for Xenopus AVT- and MT- associated neurophysins were also used.

MT and AVT cells were found in the preoptic area and posteriorly over the optic chiasm along the sulcus limitans. The region of AVT cells in Bufo extended further anteriorly into the striatum and further posteriorly into the infundibulum. TRH positive cells were found in every section examined, from the granular area of the olfactory bulb anteriorly to the trigeminal motor nucleus posteriorly and including preoptic and hypothalamic regions.

In transverse sections these distributions were distinct but had many areas of overlap and intermixing. The longitudinal extent of TRH greatly exceeded that of AVT, which exceeded that of MT. A parsimonious explanation is that longitudinal columns of neural plate progenitors are specified as to permissible peptidergic phenotypes of their progeny, and that the convoluted and intermixed adult pattern derives from subsequent cell and tissue movements overlayed upon an initially simple pattern.

359.11

INDUCTION OF MITOTIC ARREST AND MORPHOLOGIC DIFFERENTIATION OF NEUROBLASTOMA CELLS BY NEOCARZINOSTATIN: CYTOLOGIC AND THERAPEUTIC IMPLICATIONS. N. F. Schor. Division of Child Neurology, Children's Hospital, Pittsburgh, PA 15213

Neocarzinostatin is a chemotherapeutic agent which consists of a chromophore noncovalently bound to a protein. We have found that, in addition to its antimitotic effect upon C1300 neuroblastoma cells in culture, neocarzinostatin induces the morphological differentiation of these cells. Unlike the case for the antimitotic effect, this process requires the intact protein-chromophore complex. Chromophore alone or protein alone are antimitotic, but do not induce morphological change. The morphology-altering effect of neocarzinostatin, coupled with its well studied chemical mode of action may provide insight into the mechanisms underlying neural differentiation.

The activity of neocarzinostatin increases with increasing intracellular sulfhydryl content. We have increased the sulfhydryl content of C1300 cells with 6-mercaptodopamine, a compound which preferentially enters cells with catecholamine receptors. This increases the potency of neocarzinostatin for these cells. No reduction in growth rate or morphological alteration is seen with 6-mercaptodopamine alone, indicating that the effect of the sulfhydryl compound is due to facilitation of neocarzinostatin efficacy. Since 6-mercaptodopamine preferentially enters cells such as neuroblastoma cells which harbor dopamine receptors, it is hoped that this approach will lead to the development of targeted therapy for neural crest tumors.

359.12

IMMUNOHISTOCHEMICAL DOUBLE-LABELING FOR GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) AND TRANSFERRIN (Tf) PERMITS THE CATEGORIZATION OF GLIAL CELLS IN SECTIONS OF RAT BRAIN. V.K. Vijayan and S.M. Martin. Dept. of Hum. Anatomy, Univ. Calif. Sch. of Med., Davis, CA 95616.

Categorization of glial cells into subclasses is a prerequisite for evaluation of properties and functions of glia in vivo. We used double-labeling immunohistochemistry for GFAP and Tf to identify subpopulations of glial cells in rat brain. Under anesthesia, rats received cortical stab wounds and were sacrificed by transcardiac perfusion with 4% paraformaldehyde (PFO), 4% periodate-lysine-paraformaldehyde (PLP) or formalin-alcohol-acetic-acid (FAA). Paraffin sections and Vibratome slices of the brain were subjected to sequential immunohistochemical staining for GFAP and Tf and counterstained with methyl green. Three subpopulations of glia were observed in the cerebral cortex adjacent to the wound: $GFAP^+,Tf^-$; $GFAP^-,Tf^+$ and $GFAP^-,Tf^-$. No double-stained cells were detected. $GFAP^+,Tf^-$ cells resembled traditional astrocytes. $GFAP^-,Tf^+$ cells exhibited Tf staining in the cytoplasm with few reactive processes. Nuclear chromatin pattern of these cells resembled that of oligodendrocytes. $GFAP^-,Tf^-$ cells were tentatively identified as microglia. Tf staining was optimal in brains fixed with FAA, satisfactory with PLP and poor with PFO. Our studies support the use of immunohistochemical double-labeling for GFAP and Tf in identifying astrocytes and oligodendrocytes in sections of rat brain. This approach may prove useful in evaluating changes in the number of these two subclasses of glia in the rat brain following injury.

SYNAPTOGENESIS III

360.1

VIDEO MICROSCOPY OF FIRST CONTACTS IN CA1 HIPPOCAMPAL CELL CULTURES. M.W. Cooper* and S.J. Smith* (SPON: P. Forscher). Section of Molecular Neurobiology, Yale University School of Medicine. 333 Cedar St., New Haven, CT 06510

We used digital video microscopy to observe neurons establishing their first contacts in newborn rat hippocampal cell cultures. Newly dissociated cells were put in a sealed chamber at times ranging from 6 to 18 hours after plating and placed under an inverted microscope equipped with computerized autofocus and auto-scanning to sample multiple fields in long term experiments. Our previous studies using immunocytochemical stains aided in the recognition of the cell types *in vitro*.

We find that: (1) the physical interactions between cells are always mediated by filipodia arising from both interacting cell surfaces. (2) Incoming growth cones can induce protrusions from cell surfaces previously devoid of filipodia. Growth cones do this by repeatedly touching the target cell surface. (3) The contact of one filipodium triggers redirection of others to the target. Filipodial interactions are followed by either: (a) retraction of the growth cone or (b) stable contact formation brought about by accelerated growth and the coalescence of filipodia, culminating in the close apposition of the two cells. (4) Cell-to-cell contacts generate tension as evidenced by the rapid shortening of the contacting processes and consequent displacement of their cell bodies. This sequence of events is necessary for the formation of stable contacts.

Neuron-astrocyte contacts are also common and the initial interactions have many similarities to those seen in neuron-neuron contacts, although astrocyte projections are lamellar in nature.

360.2

GRADUAL LOSS OF MULTIPLE INNERVATION VISUALIZED WITH A SIMPLE CONFOCAL MICROSCOPE. J.W. Lichtman, R.J. Balice-Gordon, and W.J. Sunderland. Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

It is unknown whether the competition underlying synapse elimination causes an abrupt or a gradual loss of synapses. We have addressed this question by visualizing the transition from polyneuronal to single innervation at endplates in the transversus abdominis muscle of embryonic Elaphe snakes. Using activity dependent uptake of fluorescent molecules (Nature 314: 357-359, 1985) to separately label the synapses of different axons converging on the same endplate, we studied how terminals of competing axons are interspersed as development proceeds. In embryonic muscle, the activity-dependent labelling technique gave an unsatisfactorily high background which we compensated for by building a simple confocal attachment for our fluorescence microscope. This device reduces scattered and out-of-focus light with a series of apertures inserted into the light path at a point where each aperture is used to simultaneously illuminate and view the specimen. The adaptation is straightforward and inexpensive.

At 3-4 weeks before hatching, inputs are extensively intermingled and occupy roughly equal proportions of the developing endplate. As development proceeds, however, the inputs segregate from each other and typically one axon occupies a significantly larger area within the endplate than another. By 2 weeks before birth, this segregation becomes more pronounced as one axon's terminal area continues to grow while another axon's terminal area shrinks, often to one remaining bouton. By birth all of the endplates are singly innervated. These results suggest that the competitive interactions between convergent inputs result in the gradual elimination of one axon's terminals. The axon which ultimately persists at an endplate captures a progressively larger share of the endplate territory.

360.3

ELIMINATION AND ELABORATION OF PRE- AND POSTSYNAPTIC SITES DURING SYNAPTIC COMPETITION AT DEVELOPING NEUROMUSCULAR JUNCTIONS. R.J. Balice-Gordon and J.W. Lichtman, Department of Anatomy and Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110

We have studied the sequence of pre- and postsynaptic events during the elimination of multiple innervation at developing neuromuscular junctions in mouse sternomastoid muscle. Using 4-Di-2-ASP to vitally label motor neuron terminals and a non-blocking dose of fluorescently tagged alpha-bungarotoxin to label postsynaptic acetylcholine receptors, the same junctions were repeatedly studied at short intervals during the first postnatal month.

At multiply innervated junctions, some nerve terminal branches and the postsynatpic acetylcholine receptors underlying them are rapidly eliminated, often in less than two days. At the same junctions, other terminal and receptor sites are gradually elaborated. This elaboration continues until the end of the first postnatal month, resulting in junctions that gradually become more complex. After this time, the configuration of both nerve terminals and receptors is remarkably stable, enlarging by simple expansion as muscle fibers grow.

Thus elimination and elaboration of synaptic sites are concurrent events. Interestingly, at multiply innervated junctions, the remaining axon does not elaborate terminals at sites vacated by eliminated terminals. Eliminated terminal and receptor sites therefore represent permanent alterations in the arborization pattern of the endplate.

These results suggest that axons do not compete for occupation of the same postsynaptic sites, and that competition alters not only nerve terminals but also postysynaptic acetylcholine receptors.

360.4

PHYSICAL CHARACTERIZATION OF XENOPUS NERVE-MUSCLE AND MUSCLE-MUSCLE SYNAPTIC ADHESION IN CELL CULTURE. A.M. Brown* and M.m. Poo. Section of Molecular Neurobiology, Yale Univ. School of Medicine, New Haven, CT 06511.

When a growth cone encounters a target cell stable adhesion develops between the two cell surfaces. This mechanical connection is the first event in the structural development of the mature neuromuscular junction. Similarly, when two muscle cells encounter each other they adhere and become electrically coupled. In order to understand the early recognition events in the formation of chemical and electrical synapse we have studied the kinetics of adhesion development in these two systems.

The experiments were performed by manipulating a spherical myocyte (myoball), using a suction micropipet, into contact with another myoball or a neurite adhering to the culture substrate. After waiting a specified interval the pipet is withdrawn to test the strength of the adhering bond. We have developed a qualitative assay for neuromuscular adhesion that operates by examining the morphological characteristics of the cells during separation. Four grades of adhesion were defined based on the formation of membrane filaments connecting myoball and neurite, deformation of the growth cone, and detachment of the growth cone from the substratum. Quantitative muscle-muscle adhesion experiments are accomplished by bringing two myoballs into contact using two suction pipets. By holding one cell firmly while applying stepwise increasing suction to hold the other cell, a quantitative estimate of the critical separation force is obtained.

Results from these studies indicate monotonic increases in intercellular adhesion within 10 to 15 minutes following cell contact. This time course correlated well with the observed post contact increase in synaptic efficacy and the localization of specific cell surface components at the contact sites.

360.5

LOCAL ACCUMULATION OF ACETYLCHOLINE RECEPTORS IS INSUFFICIENT TO INDUCE CLUSTERING. J. Stollberg* and S.E. Fraser. Dept. Physiology and Biophysics, UC Irvine, Irvine, CA 92717.

Acetylcholine receptors (AChRs) aggregate at the developing neuromuscular junction by mechanisms not fully understood, but at least in part *via* lateral migration. We have shown that in a simple model system - cultured *Xenopus* muscle cells exposed to external electric fields - AChRs, concentrated at the cathode-facing cell pole, continue to aggregate there after the field is terminated (Stollberg and Fraser *J. Cell Biol.* 106, 1988). This aggregation event is due to lateral migration, sensitive to trypsin digestion, and insensitive to agents disrupting microtubules and microfilaments, suggesting that the aggregation is mediated by ad- or cohesion events on the extracellular membrane surface. The simplest model accounting for these observations is that the field-induced rise in AChR concentration triggers the aggregation event. Our present results argue against this interpretation, and suggest the aggregation involves other molecule(s), a voltage-sensitive mechanism, or both.

Treatment of myoball cultures with neuraminidase changes the charge on the cell surface, and has been reported to reverse the direction of electro-osmotic migration for AChRs and concanavalin A binding sites (Orida and Poo *Nature* 275, 1978). Using digitally-analyzed fluorescence video-microscopy, we find that the field-induced distribution of AChRs is bi-modal (density is high at the anode- and cathode-facing poles), suggesting that the electro-osmotic migration and aggregation of AChRs are opposed following neuraminidase treatment. Additionally, we find that the AChR density increases at the cathode-facing pole after field termination, but decreases at the anode-facing pole - clear evidence that elevated AChR density alone does not initiate post-field clustering. As trypsin disrupts the aggregation event, we also analyzed the AChR distribution on cells pre-treated with neuraminidase and trypsin. Consistent with the above interpretation, these cells showed elevated and decreased AChR densities at the anode- and cathode-facing poles, respectively, in a manner well-explained by electro-osmotic migration and diffusion. After termination of the field, the distribution decayed back to uniformity. The kinetics of asymmetry development with time in the field, and decay post-field, give an estimated diffusion constant for the AChR of 1.2×10^{-9} cm^2/sec.

These results indicate that AChR accumulation *per se* is neither necessary nor sufficient to trigger AChR aggregation. It appears that aggregation requires the accumulation (or perhaps voltage-sensitive action) of some other molecule(s).

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360.6

JS-1, A COMPONENT OF SYNAPTIC BASAL LAMINA AT THE NEUROMUSCULAR JUNCTION, Dale D. Hunter[1], John P. Merlie[2], and Joshua R. Sanes[1]. Dept. of [1]Anatomy & Neurobiology and [2]Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110

Axons preferentially reinnervate original synaptic sites in denervated muscle and differentiate there into nerve terminals. These phenomena are mediated at least in part by the synaptic portion of the muscle fiber's basal lamina sheath. Immunohistochemical studies have defined antigens concentrated in synaptic basal lamina which could mediate these interactions; we recently used immunoblotting and immunoprecipitation to identify one such antigen, JS (Junction-Specific)-1, a glycoprotein of Mr 185,000 and pI 5.5-6.0 (Soc. Neurosci. Abstr. 13: 375, 1987).

We have now purified JS-1 greater than 2000-fold to $\geq$20% purity. In addition, we have used monoclonal antibodies to JS-1 to isolate a cDNA clone from a λgt11 library which encodes a fragment of JS-1. Antisera raised against the encoded fusion protein recognize synaptic sites in skeletal muscle, supporting the classification of this fragment as part of JS-1. We are currently characterizing the purified protein and sequencing the cDNA.

To determine whether neurons can recognize JS-1, we plated ciliary ganglion cells (which form skeletal neuromuscular junctions in vivo) on dishes coated with purified or recombinant JS-1. Neurons adhere to either substrate, but do not send out neurites; on laminin, they adhere and send out neurites; on several control substrata, they do neither. This result is consistent with the hypothesis that JS-1 is a component of the synaptic basal lamina with which neurons interact. We are therefore asking whether neurites extending on laminin stop growing and/or differentiate when they encounter a deposit of JS-1. (Sponsored by MDA and NIH.)

360.7

MOTOR NEURONS CONTAIN AGRIN-LIKE MOLECULES DURING THE PERIOD OF NEUROMUSCULAR SYNAPTOGENESIS. C. Magill & U.J. McMahan. Dept. of Neurobiology, Stanford University, Stanford, CA 94305

Several lines of evidence indicate that the molecules in the synaptic basal lamina at the neuromuscular junction that direct the formation of acetylcholine receptor (AChR) and acetylcholinesterase (AChE) aggregates on regenerating muscle fibers are similar to agrin, a protein extracted from Torpedo electric organ. It has been hypothesized that the agrin-like molecules in the basal lamina are secreted by the motor neuron's axon terminal, that they are the same molecules that mediate the motor neuron-induced formation of AChR and AChE aggregates on developing myofibers in the embryo, and that they direct the maintenance of these postsynaptic specializations in the normal adult. Our previous studies have provided evidence that motor neurons in perinatal and adult animals contain agrin-like molecules, which is consistent with these hypotheses. The two experiments described here further revealed that agrin-like molecules are present in embryonic motor neurons at the times when AChR and AChE aggregates begin to form at neuromuscular junctions.

First, we stained the spinal cord of chick embryos with anti-agrin monoclonal antibodies. We detected anti-agrin labelling in the cytoplasm of motor neurons as early as stage 27, when neuromuscular synaptogenesis begins. The level of staining increased with developmental age at least until hatching, a period during which there is rapid synaptic growth. Motor neurons were the only neurons in which staining was detected at any age.

Second, we dissociated embryonic chick (stage 27-31) spinal cords, separated the cells into motor neuron-enriched and motor neuron-poor fractions, and made extracts of each. The extract of the motor neuron-enriched fraction contained AChR-aggregating activity which was specifically immunoprecipitated with anti-agrin monoclonal antibodies, whereas the extract of the non-motor neuron fraction contained little such activity.

360.8

AGRIN RELATED MOLECULES ARE EXPRESSED IN ANEURAL DEVELOPING MUSCLE. Justin R. Fallon and Celia E. Gelfman*, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Molecules closely related if not identical to agrin, 'agrin-related molecules', are highly concentrated in the synaptic basal lamina of the adult neuromuscular junction and are likely to play a role in directing the regeneration of the postsynaptic apparatus at this synapse. Previous work from this laboratory has established that in developing chicks agrin-related molecules are expressed in muscle prior to synapse formation and are subsequently co-localized with >95% of AchR clusters. In the present studies two preparations were used to determine if the expression of agrin-related molecules in developing muscle is dependent upon innervation. In one set of experiments, embryos with aneural hindlimbs were generated by extirpating the lumbosacral spinal cord at stage 17 (2.5d), before motoneurons have exited the CNS. In a second series, limb buds which had not yet been innervated (stage 19-21, 3.5d), were removed from embryos and grafted onto the chorioallantoic membrane. Seven days after either manipulation the experimental limbs were examined by fluorescence microscopy of cryostat sections using antibodies against agrin and rhodamine α-bungarotoxin. In both cases agrin related molecules are present in the aneural muscles and co-localize with AchR clusters. These experiments extend our previous results which indicate that agrin-like molecules play a role in the initial events of synapse formation and also suggest that at least some of the agrin-like molecules present at the neuromuscular junction are synthesized by muscle.

360.9

TARGET RECOGNITION DURING SYNAPTOGENESIS OF CULTURED *HELISOMA* NEURONS. Zoran, M. J. and Haydon, P. G. Department of Zoology, Iowa State University, Ames, IA 50011

Cholinergic neuron B5, isolated from the buccal gangion of *Helisoma* and plated into culture, reliably forms the presynaptic element of inappropriate chemical synapses with ACh-sensitive, identified neurons P5, B19 and B5. In contrast, cholinergic neuron B19 is unable to form chemical connections with the same inappropriate, postsynaptic targets. Is recognition of an appropriate postsynaptic cell necessary for B19 to become an adequate presynaptic element?

Single supralateral radular tensor (SLT) muscle fibers, known to be the postsynaptic elements of cholinergic connections with neuron B19 *in vivo*, were dissociated into cell culture. Single muscle fibers maintain normal physiological properties in cell culture; application of ACh evokes depolarizing potentials and contractile responses. Neuron B19 was plated in cell culture with the single SLT target fibers and, after a period for neurite extension and contact, formed cholinergic chemical synapses. Thus, B19 is capable of forming appropriate chemical synapses when plated with its appropriate target.

Therefore, although both neurons B19 and B5 can form chemical synapses in culture, they utilize different synaptogenic strategies. Neuron B19 requires the recognition of an appropriate target for synapse formation; whereas, B5 forms inappropriate synapses with any ACh-receptive target.

This work was supported by NIH grant NS24233.

360.10

RECEPTOR-MEDIATED REGULATION OF SYNAPTIC CONNECTIVITY IN CULTURED *APLYSIA* NEURONS. G.M. Carrow and I.B. Levitan. Graduate Department of Biochemistry, Brandeis Univ., Waltham, MA 02254.

When dissociated neurons from the mollusc, *Aplysia californica*, are placed in primary cell culture, they form electrical synapses in a specific manner. Neurons from the same ganglion form electrical synapses (homoganglionic synapses) with high coupling coefficients due to high macroscopic junctional conductance (G_j; 10-25 nS). By contrast, synapses between neurons from different ganglia (heteroganglionic synapses) exhibit low coupling coefficients as a result of low G_j (1-5 nS). Moreover, this specificity is altered by treatment of the neurons with the lectin, Concanavalin A (Con A; Carrow & Levitan, 1987; Lin & Levitan, 1987).

We have now found that the alteration in synaptic specificity induced by Con A results from its increasing the junctional conductance of heteroganglionic synapses to the level characteristic of homoganglionic synapses. Furthermore, this increase in conductance is dependent upon protein synthesis. Pairs of neurons isolated from juvenile ganglia and grown in culture in the absence of Con A were voltage clamped and perfused with 0.1 μM Con A. The G_j of heteroganglionic pairs began to increase about 2 hr after introduction of Con A and continued to increase over several hours, even after wash out of the lectin. Homoganglionic pairs treated similarly showed no change in G_j. Heteroganglionic pairs failed to show increased G_j when treated for 1 day with 0.5 μM Con A in the presence of the protein synthesis inhibitor, anisomycin (10 μM). However, this blockage was partially reversible since G_j increased several fold in the continued presence of Con A after wash out of anisomycin.

Synaptic specificity among these regenerating neurons is likely mediated by ganglion-specific cell recognition molecules. The results summarized here suggest that these molecules are or are linked to lectin receptors that regulate synthesis of gap junction channels or associated regulatory proteins.

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360.11

GLUTAMATE RELEASED FROM ENTORHINAL CORTEX AXONS INHIBITS DENDRITIC OUTGROWTH AND PROMOTES SYNAPTOGENESIS IN HIPPOCAMPAL NEURONS. M. P. Mattson, R. E. Lee*, M. E. Adams*, P. B. Guthrie & S. B. Kater, Program in Neuronal Growth & Dev., Colorado State Univ., Ft. Collins, CO 80523.

A culture system of embryonic rat entorhinal cortex explants and isolated hippocampal neurons was used as a model of hippocampal development to demonstrate that endogenously-released neurotransmitters can shape neuronal circuitry. Hippocampal neuron dendrites were visualized by MAP2 immunocytochemistry on the background of nonstaining entorhinal axons; little dendritic outgrowth occurred in neurons on the axon bed. Since glutamate can inhibit dendritic outgrowth in isolated hippocampal neurons (Mattson et al., J. Neurosci. 8:2087, 1988), we hypothesized that glutamate, released from entorhinal axons, might be suppressing dendritic outgrowth. HPLC analysis of culture medium showed activity- and calcium-dependent, release of glutamate from explants. The glutamate receptor antagonist d-glutamylglycine (DGG) significantly increased dendritic outgrowth in hippocampal neurons on the axon bed. Tetrodotoxin, elevated extracellular Mg^{2+}, or removal of entorhinal cell bodies also enhanced dendritic outgrowth; apparently ongoing activity in entorhinal neurons and associated glutamate release was responsible for outgrowth inhibition. Both the geometry and synaptic organization of a neuron determine its information coding capabilities. We therefore examined synaptic correlates of glutamate's effects at the light (Timm method and protein III staining) and ultrastructural levels. In control cultures synaptic specializations associated with the soma and dendrites of hippocampal neurons were abundant and well differentiated containing numerous small (30-50 nm) vesicles. In contrast, synapses in DGG-treated cultures were reduced in numbers and were poorly developed with relatively few, large (>100 nm) and irregular-shaped vesicles. The mechanism by which glutamate inhibits outgrowth and promotes synaptogenesis apparently involves sustained elevations in intracellular calcium; DGG reduced intracellular calcium levels in hippocampal neurons on the axons. Our data indicate that neurotransmitters can shape both the neuritic architecture and specific connections of the circuits in which they participate in information coding. (MPM is a French Foundation for Alzheimer's Disease Fellow).

ALZHEIMER'S DISEASE: PROTEIN

361.1

Reduction of APP-695 Beta-Amyloid Transcript Prevalence in Alzheimer Disease (AD) Cortex. SA Johnson, GM Pasinetti and CE Finch, Andrus Gerontology Center and Dept. Biol. Sci., Univ. of So. Cal., Los Angeles CA, 90089-0191

Recent work has shown that mRNA for the beta-amyloid precursor protein (APP) exists in the brain in two differentially spliced forms (APP-695 and APP-751). APP-751 has an additional exon encoding a "Kunitz serine-protease inhibitor" peptide (Ponte et.al., Nature 331:525, 1988). We examined whether the different beta-amyloid transcripts have different prevalence in AD or control (CTL) cortex, using probes and hybridization conditions to discriminate APP-695 and APP-751 transcripts separately on poly(A)+RNA gel blots. In initial studies with pooled cortical A+RNA from AD or CTL (5 individuals per pool), two closely migrating bands were detected at 3.6 Kb & 3.4 Kb (APP-751 probe) and at 3.4 Kb & 3.2 Kb (APP-695 probe). Mixing studies showed each probe to be highly selective and consistent with alternate polyadenylation sites. Autoradiogram quantitation indicated a 65% decrease in AD for the APP-695 transcript doublet. Overall, the ratio of APP-751/APP-695 increased 2 fold in AD. Similar results were obtained with individual A+RNA samples (5 AD and 4 CTL). While the prevalence of the APP-751 full length doublet did not change in AD, the APP-695 transcripts decreased 60%. The APP-751/A-695 transcript prevalence ratio increased from 2.4 in CTL to 5.6 in AD. We also detected a new 1.6 Kb APP-695-related transcript in both AD and CTL A+RNA, whose prevalence with respect to full-length APP-695 transcripts increased 3-fold in AD. In situ hybridization experiments are in progress to determine whether different neuronal types show selective loss of these transcripts in AD. The large decrease of transcripts lacking the Kunitz serine protease inhibitor peptide could be important to AD pathogenesis by disturbing the balance of protein processing and degradation. These studies were supported by grants to CEF (AG-05142, ADRC of Southern California) and to SAJ from ADRDA.

361.2

AMYLOID PROTEIN PRECURSOR mRNAs: DIFFERENTIAL EXPRESSION IN ALZHEIMER'S DISEASE. M. Palmert*, T. Golde*, M. Cohen*, D. Kovacs*, R. Tanzi*, J. Gusella*, M. Usiak*, L. Younkin, and S. Younkin. Case Western Reserve and Harvard Schools of Medicine.

We used *in situ* hybridization (ISH) with [35]S-labeled cRNA probes to assess amyloid protein precursor (APP) mRNA in 11 sporadic Alzheimer's disease (AD) and 7 control brains well matched for age and postmortem interval. Total APP mRNA was evaluated using a 3' probe complementary to all APP mRNAs. APP mRNA containing the Kunitz protease inhibitor (KPI) domain was separately evaluated using a probe complementary to the KPI sequence. In each experiment, sets of AD and control sections from the same brain region were processed side-by-side to optimize comparison of mRNA levels in AD and control cases. In AD we observed a significant two-fold increase in total APP mRNA in nucleus basalis of Meynert (nbM) and locus ceruleus (LC) neurons, but there was no change in total APP mRNA in hippocampal subicular neurons, neurons of the basis pontis, or occipital cortical neurons. With the KPI-specific probe, we observed no change in any of the brain regions examined. Thus the increase in total APP mRNA in nbM and LC neurons was due exclusively to an increase in APP mRNA lacking the KPI domain. We are currently performing ISH with junctional probes in an effort to confirm the selective increase in the APP mRNA lacking the KPI domain, to assess the basal level of expression of this form in control cells, and to determine if there is a large percentage increase in this form in AD. (Supported by USPHS grants AG06656, MH43444, and a grant from the state of Ohio.)

361.3

THE MONKEY HOMOLOG OF THE β-AMYLOID PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE: ISOLATION OF cDNAs AND DETECTION OF PRECURSOR PROTEIN. M. Berman-Podlisny,* D. Tolan* and D. Selkoe (SPON: G. Strichartz). Harvard Med. Sch., Brigham and Women's Hosp. and Boston University, Boston, MA.

Alzheimer's disease is always accompanied by the deposition of extraneuronal filaments composed of the β-amyloid protein (βAP). A similar process occurs during normal brain aging in humans and lower primates. We have shown that amyloid in aged monkey cortex contains βAP antigenically indistinguishable from that in AD. Thus, aging monkeys may prove useful for dynamic studies of the processing of the βAP precursor (βAPP) into amyloid that are not possible in humans. Human βAPP cDNAs were used to probe a cDNA plasmid library from adult monkey cortex. Of ~60 clones detected, the longest insert was ~1.9 kb. To date, we have sequenced 0.8 kb, yielding a monkey sequence that shows 97.7% nucleotide homology in the region 1279 - 2073 bp of the human sequence (Kang et al., Nature 1987). The nucleotide changes produced no amino acid substitutions within this 264-residue region that includes the βAP. Antibodies to C-terminal synthetic peptides of the human βAPP were used to probe Western blots of monkey brain. We detected proteins of ~110-135 kDa that co-migrate with polypeptides we have identified as forms of the native βAPP in human tissues. These comparative studies should enable analysis of the proteolytic processing of the βAPP in a non-human primate that undergoes cortical amyloidosis and neuritic plaque formation with age.

361.4

Isolation and expression of multiple forms of Beta Amyloid Protein pecursor cDNAs. R.J. Donnelly*, C. G. Rasool, R. Bartus, B. Beer, A.J. Blume, and M. Vitek. Molecular Neurobiology and Geriatrics, Central Nervous System Biological Research Department, Medical Research Department, Lederle Laboratories, American Cyanimid Company, Pearl River, NY 10965.

Alzheimer's Disease (AD) is associated with the extra-normal accumulation of a 42 amino acid beta-amyloid peptide (BAP) in amyloid plaques and cerebrovascular deposits. Though BAP is deposited exclusively in brains of AD and Downs syndrome patients, the mRNA encoding BAP is found in the brain and in peripheral tissues. Using an HL 60 cDNA library, we have isolated two Amyloid Peptide Precursor (APP) cDNAs with sequences that code for proteins containing 751 and 770 amino acids (APP 751 and APP 770). In addition to containing the coding region for BAP, they also contain a region homologous to the Kunitz domain of serine protease inhibitors. In order to examine the potential function of these putative precursor proteins and to study the processing of APP 751 and APP 770 into the 42 aa peptide, we have engineered the cDNA sequences into bacterial and eucaryotic expression vectors. The results of the expression of the exogenous gene in mammalian cell cultures and the characteristics of the bacterially produced protein will be discusssed with respect to a possible precursor-product relationship in AD.

361.5

THE BIOLOGICAL ACTIVITY OF ALZHEIMER'S AMYLOID. B. A. Yankner[1], L. Villa-Komaroff[1] and R. L. Neve[2]. Depts. of Neuroscience[1] and Genetics[2], The Children's Hospital, Boston, MA 02115

The rat pheochromocytoma cell line PC12 differentiates into a neuronal cell type in response to nerve growth factor (NGF). Northern blot analysis demonstrated that the amyloid precursor RNA is expressed constitutively in PC12 cells but is induced about 100-fold by treatment with NGF. cDNAs corresponding to the amyloid precursor or a fragment containing the amyloid beta protein were transfected into PC12 cells by the calcium phosphate method. Several clones were isolated that overexpressed either amyloid beta protein or amyloid precursor RNA from the transfected cDNAs. Clones overexpressing the amyloid beta protein exhibited a significant acceleration of neurite outgrowth in response to NGF. Clones overexpressing the amyloid precursor also exhibited an increased response but relatively less than cells expressing similar amounts of the amyloid beta protein fragment. Conditioned medium from transfected cells greatly potentiated neurite outgrowth when added to untreated PC12 cells or cultured dorsal root ganglion neurons. These results suggest that proteolytic cleavage of the Alzheimer's amyloid precursor may generate a peptide with potent effects on neuronal differentiation.

361.6

DEVELOPMENT OF A GENETICALLY ENGINEERED CELLULAR MODEL FOR THE AMYLOIDOSIS OF ALZHEIMER'S DISEASE. **S.B. Zain*, W.-G. Chou*, R.E. Majocha*, and C.A. Marotta (SPON: G. Hauser). Univ. Rochester Cancer Ctr., Rochester, NY 14642; Harvard Med. Sch., Massachusetts General Hosp., Boston, MA 02114; Mailman Res. Ctr., McLean Hosp., Belmont, MA 02178.**

The A4 domain of the amyloid precursor protein (APP) cloned directly from Alzheimer's Disease (AD) brain mRNA has the same structure as from non-AD sources (Zain, et al, P.N.A.S. 85:929, 1988). Local processing of the precursor may be a critical event in the formation of amyloid plaques. Therefore we prepared genetically transformed cells that overexpress the A4 domain. Transfections were carried out with synthetic vectors, originally derived from SV40, that were linked to the 1.1 kb cDNA Eco RI fragment that contains the A4 region. Successful transfection was confirmed by Southern blots and in situ hybridizations. Cells were immunostained by monoclonal antibodies to the A4 peptide. Transfectants carrying vectors in the correct reading frame for APP produced immunologically detectable amounts of the A4 epitope. The cells are being used to examine the consequences of amyloid over-production and to define conditions that interrupt this process. Supported by AG02126, CA11198, CA36432, American Health Assistance Foundation and Familial A.D. Res. Foundation.

361.7

IDENTIFICATION OF NEWLY SYNTHESIZED BETA-AMYLOID PRECURSOR PROTEINS IN RAT AND HUMAN. W. Wallace, M. Krishnamurthi J. Anderson [1], P. Mehta [2], V. Haroutunian[1], N. K. Robakis[1] Dept. of Psychiatry and Fishberg Center for Neurobiology Mt. Sinai School of Medicine, New York and [2] Institute for Basic Research in Developmental Disabilities, New York.

The amyloid core protein (ACP) likely derives from a larger protein termedthe beta-amyloid precursor protein (B-APP). Three splicing variants of the mRNA coding for B-APP have been isolated. We are using polysomes to identify and characterize the newly synthesized forms of B-APP in rat and human brain, in the absence of post-translational modifications such as those responsible for the formation of ACP. Polysomes are translated in vitro and the translation products immunoprecipitated with antibodies generated to synthetic peptides made to various portions of the putative precursor. We observe three different polypeptides (Mr 110,115,120 kD) synthesized by the polysomes, immunoprecipitated by four different antibodies, and specifically competed away by the synthetic peptides. In the human, the 115 and 120 kD species are present in considerably higher abundances relative to the 110 species compared with the rat. These same polypeptides are synthesized by cerebellar as well as cerebral cortical polysomes. All three polypeptides are made exclulsively by membrane-bound polysomes. The different newly synthesized B-APP's may be products of the mRNA splicing variants.

361.8

TAU PROTEIN IN ALZHEIMER'S DISEASE, AGED RATS AND SYSTEMIC ORGANS. S.Ch. Papasozomenos and L.I. Binder. Dept. of Pathology, Univ. of Texas Med. Sch., Houston, TX 77225 and Depts. of Cell Biol. and Neurology, Univ. of Alabama, Birmingham, AL 35294.

We have shown that in Alzheimer's disease (AD) excessive amounts of tau immunoreactivity localized not only with paired helical filaments but also with ribosomes in both neurons and astrocytes. We have also shown (Papasozomenos, S.Ch. and Binder, L.I., Cell Motil. Cytoskel. 8:210, 1987) by using two monoclonal antibodies against tau, Tau-1 and Tau-2, that phosphorylated Tau-1 epitope and the Tau-2 epitope are present on microtubules and ribosomes in the somatodendritic compartment of neurons and in astrocytes. In order to gain further insight into the physiologic functions of tau and the etiopathogenesis of AD, we have examined the tau immunoreactivity in almost every systemic organ and the CNS of several Sprague-Dawley rats 6 weeks to 25 months old. Among the systemic organs, tau immunoreactivity was detectable only in plasma cells, the islets of Langerhans and the renal medullary collecting duct epithelium. In the CNS, using the Tau-1 antibody, tau immunoreactivity was not detectable in the somatodendritic compartment of neurons and astrocytes without prior dephosphorylation of sections up to the age of 3 months, but in older rats tau non-phosphorylated at the Tau-1 epitope, and thus detectable without prior dephosphorylation of sections, appeared, besides axons, in protoplasmic astrocytes. These astrocytes were most numerous in cortical layer IV, the inferior colliculi, the granule cell layer of cerebellum, the CA3 region of hippocampus and the dorsal half of the lumbar spinal cord. Involvement of astrocytes throughout the brain and perhaps earlier than neurons was also observed in AD. These findings suggest that a disturbance of a secretory process in which tau may play a role may be involved in the pathogenesis of aging and AD.

361.9

CONCOMITANT PRESENCE OF UBIQUITIN AND TAU PROTEIN IN EXPERIMENTAL NEURONAL INCLUSIONS.

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Several diseases of the nervous system, such as Alzheimer's, Pick, Parkinson and amiotrophic lateral sclerosis are charachterized by the presence of intraneuronal inclusions whose composition and mode of formation are unknown. We have induced the formation of intracytoplasmic neuronal inclusions exposing rat dorsal root ganglia to the antimitotic drug cytosin-arabinoside (5*10e-6 M), for 7 days. The inclusions are round, approximately the size of the nucleus and markedly eosinophilic. Ultrastructurally they are composed of packed filaments mixed with trapped cytoplasmic organelles. The inclusions immunoreact with antibodies to Ubiquitin, Tau proteins and phosphorilated epitopes of the 200 kDa neurofilament subunit, but not with antibodies to actin and Heat Shock Proteins 70 and 28. Antitubulin antibodies react only with some inclusions. The experimental inclusions we have obtained may provide a model to study the mode of formation of those neuronal inclusions that form in the course of human neurodegenerative conditions and that contain Ubiquitin, Tau proteins and phosphorilated epitopes of neurofilaments.

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361.10

THE ALZ50 EPITOPE IS IN THE CARBOXY HALF OF TAU AND BACTERIAL SYNTHESIS OF THE SITE IS NOT SUFFICIENT FOR RECOGNITION.

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Alz50 is a monoclonal antibody directed against an antigen that is present at higher levels in Alzheimer versus control brain (Wolozin et al., SCIENCE 1986). Recently, we have shown that the antibody reacts with tau in all of its heterogeneous forms (Nukina et al., NEUROSCI LETT 1988). We therefore sought the epitope in tau wtih which the antibody reacts. Alz50 does not react with any of the epitopes presented to it in bacterially synthesized tau, thus suggesting the antibody is directed against a post-translational modification or a conformation not achieved in the bacterial system. Bovine tau was digested with cyanogen bromide (CNBr) and the HPLC-separated fragments tested for Alz50-reactivity. The reactive fragments eluted over a broad range with a late retention time relative to the other CNBr fragments. Tubes with Alz50-reactivity contained two tau peptides, both of which derived from the carboxy half of the molecule. The carboxy half of tau is likely to contain the microtubule binding domain. One of the peptides is likely to contain the Alz50 epitope, which may represent a site on tau that is involved in the early stages of neurofibrillary tangle formation.

361.11

IN SITU MAPPING OF pADHC-9: A POLY(A)RNA SEQUENCE OVEREXPRESSED IN ALZHEIMER'S DISEASE HIPPOCAMPUS.

P.C. May, Ph.D., S.A. Johnson, Ph.D., M.E. Lampert-Etchells, Ph.D.* and C.E. Finch, Ph.D. Andrus Gerontology Center, Dept. Biological Sciences, USC, Los Angeles, CA 90089.

Poly(A)RNA sequences with altered prevalence in Alzheimer's disease (AD) are being cloned to identify the molecular events associated with neuronal degeneration and regeneration occurring in the AD hippocampus. Clone pADHC-9 is a 0.7 Kb cDNA clone isolated from a mixed AD/Control hippocampal cDNA library by differential screening with AD and control cDNA probes. By Northern analysis, pADHC-9 hybridizes to a 2.0 Kb RNA whose prevalence was selectively increased two-fold in AD hippocampus; no increase in this RNA was detected in AD RNA samples from other brain regions which undergo degeneration (e.g., frontal, occipital or temporal cortex). To map the cellular distribution of the RNA encoded by pADHC-9, sense and antisense ^{35}S-labelled cRNA probes were hybridized to coronal sections of AD and control hippocampal formation. Direct autoradiography of tissue sections after in situ hybridization indicated a laminar distribution of the RNA encoded by pADHC-9 in the entorhinal cortex and hippocampus. Prominent labelling was observed in the granule cell layer of the dentate gyrus and along the pyramidal tract of the hippocampus. Sequence data analysis of pADHC-9 indicated no significant homology with any entry in GenBank. Additional cDNA clones nearly spanning the entire 2 Kb RNA have been obtained and are being sequenced. Provisionally we conclude that pADHC-9 represents a new marker reflecting altered gene expression in AD hippocampus. Identification of this RNA sequence and characterization of the function of its protein product will establish a new approach to basic mechanisms of pathogenesis in AD.

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361.12

ALTERATION IN GLIAL FIBRILLARY ACIDIC PROTEIN mRNA CONTENT IN THE RAT HIPPOCAMPUS AFTER ENTORHINAL CORTEX LESION.

J. Poirier*, P.C. May and C.E. Finch. Department of Neurobiology/Gerontology, Univ. Southern California, Los Angeles, CA 90089.

To better reveal the role of denervation on the hippocampal expression of selected mRNA in Alzheimer's disease, we used a rodent model of hippocampal deafferentation. Electrolytic lesions of the entorhinal cortex were made in twenty 3 month old Fisher rats. After 14 days, rats were sacrificed and total hippocampal RNA was isolated by the guanidinium isothiocyanate method of Chirgwin at al. 1979. The total RNA was used for solution hybridization and northern blot hybridization. The loss of entorhinal input in the dentate gyrus in lesioned rats results in sprouting of the afferents originating from the septal area (cholinergic) and from the commissural/ associational (glutamatergic) fibers. This plastic response is very similar to what has been observed in the hippocampus of Alzheimer's patients (Geddes et al, 1985). Based on this striking similarity of response, we are studying the expression of mRNA of proteins that were shown to be increased, decreased or associated with tangles in the hippocampus of Alzheimer's disease patients. These include glial fibrillary acidic protein, beta amyloid, somatostatin and ubiquitin. Complementary RNA (cRNA) of each of these probes were synthesized and hybridized to total RNA extracted from lesioned and control rat hippocampi. These lesions had no affect effect on total and A+ RNA yield. Ubiquitin, somatostatin and beta amyloid mRNA content in the hippocampus of lesioned animals do not differ from control animals whereas GFAP mRNA shows a highly significant increase in northern blot analysis using a computer based image densitometry. Subsequent quantification of GFAP messenger by RNA-RNA solution hybridization indicate a 3.0 fold increased expression in the hippocampus of lesioned (10.8 $\pm$ 1.8 pg/ug of total RNA) versus control (3.8 $\pm$ 0.2 pg/ug of total RNA) animals. This is in agreement with a recent report that has shown a two fold increase GFAP mRNA content in the hippocampus of patients with Alzheimer's disease (May et al., 1987). By in situ hybridization with an S35-GFAP cRNA, unilaterally lesioned animals show increased signal in the outer two thirds of the molecular layer of the dentate gyrus. It is also in the same area that maximal sprouting of cholinergic septal fibers and glial proliferation have been previously observed (Scheff et al., 1980). These results indicate that this rat model of hippocampal deafferentation can effectively mimic some of the molecular responses observed in Alzheimer's disease.

Supported by the John D. and Catherine T. MacArthur Foundation Research Program On Successful Aging.

VISUAL CORTEX V

362.1

STUDIES OF PRIMATE VISUAL CORTEX USING A DOUBLE-LABEL DG TECHNIQUE AND COLOR AUTORADIOGRAPHY.

R. B. H. Tootell, R. T. Born* and S. L. Hamilton*. Dept. Neurobi., Harvard Med. Sch., Boston, Ma. 02115.

Several investigators have experimented with a double-label deoxyglucose (DG) technique [Livingstone and Hubel, *Nature 291:* 554 (1981); Webster et al, *Neurosci. Lett. 40:* 281 (1983); Friedman et al, *Exp. Brain Res. 66:* 543 (1987)]. The potential advantage of such a technique is that brain activity produced by both an experimental and control condition can be labelled in the same tissue, typically with ^{3}H-DG in one condition and with ^{14}C-DG in the other. Following Kronenberg *[Anal. Biochem. 93:* 189; (1979)], who tested radioactive standards on color-negative film, we developed a double-label DG protocol incorporating color film autoradiography, in which the distributions of ^{3}H- and ^{14}C-DG can be reliably separated from each other. In this protocol, ^{3}H-DG produces a blue image on color-positive film, and the ^{14}C-DG produces a whitish image on the same film.

To validate our modified double-label technique, a paralyzed, anesthetized macaque monkey was shown a split-field visual stimulus, monocularly. Initially, ^{3}H-DG was injected while the animal viewed a black-white square wave grating (of systematically varied orientation, spatial frequency, drift rate and direction) confined to the lower visual field. After 45 minutes, when most of the ^{3}H-DG was taken up, the stimulus was reversed so that the grating was in the upper visual field, and ^{14}C-DG was injected to label its effect. Color autoradiographs from this case showed blue ocular dominance strips in dorsal striate cortex, whitish ocular dominance strips in ventral striate cortex, and a sharp retinotopic border between the two regions. By using blue and red color filters and digital subtraction routines, images of these ^{3}H- and ^{14}C-DG labelled ocular dominance strips could be unambiguously isolated. In another experiment, horizontal and vertical (blue and whitish) orientation columns were labelled in areas V1, V2 and other regions of macaque visual cortex with binocular, single-orientation stimulation. This technique should be extremely useful in studies of extrastriate cortical columns, where it is necessary to label the effect of at least two different stimulation conditions to prove the existence of functional columns.

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362.2

INFRA-RED IMAGING OF FUNCTIONAL ORGANIZATION OF VISUAL CORTEX THROUGH THE INTACT DURA USING A CCD CAMERA.

R.D. Frostig, E.E. Lieke, D.Y. Ts'o and A. Grinvald. IBM Research Division, Yorktown Heights, NY 10598 and Lab. of Neurobiology, The Rockefeller University, New York, NY 10021.

We have demonstrated that activity dependent intrinsic signals can be used for optical mapping of the functional organization of the visual cortex. Exploration of the nature of the intrinsic signals useful for functional imaging revealed two major components. One component originates from changes in blood volume probably due to local capillary recruitment in an activated area. These changes are followed by an increase in hemoglobin absorption. Detailed characterization of the time course and saturation of this optical signal may help to improve the performance of PET imaging which is based on similar phenomena (Fox et. al. Nature, 323, 806-809, 1986). The other component originates from light scattering that accompanies changes in the cortical tissue after activation (e.g., ion and water movement, expansion and contraction of extracellular spaces, capillary expansion, neurotransmitter release, etc.).

The blood volume component of the intrinsic signal dominates between 500-700 nm while the light scattering component dominates in the near infra-red region above 800 nm. These two components have different time courses. Nevertheless, maps of functional organization obtained in both regions of the spectrum were identical either in area 17 of the macaque monkey or area 18 of the cat. Thus each component can be used for functional mapping (see also Ts'o et. al. this volume).

We have found that the proper control of depth of field is an important factor in optical imaging. With present optics the depth field is probably larger than 500 μm. Since near infra-red light penetrates the cortex deeper than visible light it facilitates the imaging of deeper cortical structures. Thus infra-red imaging may prove more useful for confocal three-dimensional imaging than visible light. Moreover, near infra-red light also allowed us to image, using a high resolution CCD camera, the orientation columns of the cat and the ocular dominance columns of the monkey through the intact dura, which is opaque to visible light. The obtained maps were identical to maps obtained after the dura was removed. The ability to image the functional organization through the dura will be advantageous for research on chronic animal preparations.

362.3

FUNCTIONAL ORGANIZATION OF VISUAL AREA 18 OF MACAQUE AS REVEALED BY OPTICAL IMAGING OF ACTIVITY-DEPENDENT INTRINSIC SIGNALS *D.Y. Ts'o, R.D. Frostig, E.E. Lieke, A. Grinvald. Laboratory of Neurobiology, The Rockefeller University, NY, NY 10021 and IBM Research Division, Yorktown Heights, NY 10598.*

Using a high *S/N* CCD camera, we have extended the spatial resolution of the imaging of intrinsic activity-dependent optical signals[1] (i.e. without dyes), and applied the technique to study the functional organization in the macaque visual areas 17 and 18. These methods have enabled us to directly image *in vivo* the thick and thin cytochrome oxidase-rich stripes as well as other functional features of area 18.

By presenting visual stimuli to each of the two eyes separately and subtracting the two sets of frames after averaging, the familiar pattern of ocular dominance in area 17 was obtained. However this procedure revealed no pattern in area 18. In an alternative strategy, the same two sets of frames were each compared to a set of no-visual-stimulus frames. This method revealed stripe-like cortical regions activated by each of the two monocular stimulus conditions that were overlapping in area 18 rather than interdigitating as in area 17. Comparison with cytochrome oxidase histology from the same tissue showed that these regions of higher activity imaged optically coincided with the thick and thin stripes of area 18. A similar overlapping of regions of activity with monocular stimulation of each of the two eyes has been seen using the double-label 2DG method.[2] We later discovered that frames obtained with binocular stimulation also showed the stripes in area 18. The stripes imaged optically, then, may represent a co-mingling of cells from both ocular dominance groups, or perhaps regions that have high responsivity or low stimulus selectivity.

We also used these techniques to map orientation specificity in areas 17 and 18. The resultant patterns appeared as an arrangement of patches that were, in area 17, 200-400μm in diameter, but in area 18 were 500-800μm, showing large regions with uniform orientation selectivity. This feature of the organization of orientation selectivity in area 18 has been suggested by single unit recordings in area 18.[3]

[1] Grinvald et al, Nature *324:* 361-364. [2] Bruce, Friedman and Goldman-Rakic, Soc.Neurosci.Abstr. *11:* 1089. [3] Hubel and Livingstone, J.Neurosci *7:* 3378-3415.

362.4

PATHWAY TRACING IN PRIMATE VISUAL CORTEX USING VOLTAGE SENSITIVE DYES IN VIVO. H. S. Orbach, D. J. Felleman and D. C. Van Essen. Div. of Biol., Caltech, Pasadena, CA 91125.

We have used voltage-sensitive dyes in conjunction with focal electrical stimulation to map connections between visual areas in the anesthetized squirrel monkey. Dorsal V1 and V2 were exposed, stained with a styryl dye (RH795, courtesy A. Grinvald and R. Hildesheim), and imaged onto a 10 x 10 photodetector array. Area V1 was stimulated with a brief train of pulses passed through a tungsten microelectrode. Large responses were seen with current pulses as low as 10 uamp x 0.2 ms (biphasic).

Within V1, local responses extended several mm. from the stimulation site; the full-width at half-height was 1-2 mm. Responses in the periphery of this zone began 4 msec or more after those in the center, which presumably reflects conduction and/or synaptic delays.

In two hemispheres, responses were seen at the expected topographic location in V2. These were well separated from the V1 stimulation site and began with a delay of 5-8 msec. The region of activation in these instances included all three cytochrome-oxidase stripes (thick, thin and inter-stripe). Thus, this paradigm should be valuable for probing the topographic (and probably compartmental) organization of cortical visual areas. Supported by the Office of Naval Research.

362.5

SPATIAL FREQUENCY ORGANIZATION OF PRIMATE STRIATE CORTEX D.H. Grosof*, M.S. Silverman*, R.L. De Valois* and S.D. Elfar*.(SPON:D. Bentley) Dept. Psychology, Univ. of California, Berkeley, CA 94720

We measured the spatial frequency (SF) tuning of cells at regular intervals along tangential probes through the supragranular layers of the monkey striate cortex, and correlated the recording sites with the cytochrome oxidase patterns, to address three questions with regard to the cortical SF organization:

1. Is there a periodic anatomical arrangement of cells tuned to different spatial frequencies? Yes. The preferred SF in successive loci changes systematically: it gradually rises and falls. The cycle repeats with a period of about 0.6 to 0.7 mm.
2. Are there just two populations of cells, low SF and high SF, at a given eccentricity, or is there a continuum? There is a continuum, with most tuned to the intermediate SFs, forming a unimodal distribution. Further, the cells with different peaks were found to be continuously and smoothly distributed across a module.
3. What is the relation between the physiological SF organization and the cytox blobs? We found cells with different spatial frequency tuning to be systematically mapped onto the modular cytox pattern, which varies continuously in staining density. Low SF cells are at the center of the blobs and cells tuned to increasingly higher SFs are at increasing radial distances.

362.6

FUNCTIONAL ORGANIZATION OF THE BLOB CELLS IN THE MONKEY STRIATE CORTEX. Charles R. Michael, Dept. of Cellular/Molecular Physiology, Yale School of Medicine, New Haven, CT. 06510.

Closely spaced, perpendicular tungsten electrode tracks were made through the cytochrome oxidase blobs in the supragranular layers of the monkey's striate cortex to examine the relationships of the various cell types to one another. Double opponent color cells seemed to be concentrated in the centers of the blobs; broad band units were found primarily at the edges; and in between, there were Type II and modified Type II neurons. Double opponent cells were maximally excited by presenting one color in the center while simultaneously illuminating the surround with the complementary color. Spectral sensitivity curves revealed that the surrounds were organized in an opponent color manner. Some of these cells, particularly in layer 2, did not respond to colored annuli. The modified Type II cells never discharged to annular stimuli. Their surrounds had broad-band spectral sensitivities, thus preventing any response to large spots or two-colored stimuli. Type II cells were often seen in layer 3 while modified Type II neurons were more prevalent in layer 2. Supported by NIH Grant EY 00568.

362.7

A COMPUTER SIMULATION OF CORTICAL ORIENTATION SELECTIVITY IN THE CAT VISUAL SYSTEM. U. J. Wehmeier and C. Koch, Division of Biology, 216-76, Caltech, Pasadena, CA 91125.

The manifestation of orientation tuned responses in cells of cat visual cortex has been addressed by models utilizing excitatory feedforward connections (Hubel & Wiesel,1961) and more recently utilizing intracortical inhibition (Heggelund,1981; Silito,1975). Recently, one of us has proposed an ecclectic model of orientation selectivity, combining aspects from all these models (Ferster and Koch, 1987). We have implemented a computer simulation of a patch of the foveal X system of the cat based on anatomical and physiological data to evaluate these different models. The simulation demonstrates responses of a 2x2 degree patch of visual angle in the retina, its projection to LGN, and its subsequent projection to layer IVc in cortical area 17. The retinal image is modeled using gaussian sensitivity profiles with different constants for center and surround fields (Linsenmeier et al., 1982), and is then sampled on a hexagonal grid of beta cells (Wässle et al., 1981).

We have found that two types of inhibitory projections, cross-orientation and inhibition among similar oriented cells with spatially displaced receptive fields, superimposed on a Hubel and Wiesel type scheme, to be effective in discriminating orientation. Due to the massive inhibitory feedback among our 2500 cortical cells, each cell can be highly orientation selective and no non-oriented interneurons are required. Simulation results indicate that presentation of low contrast bars, while not being discriminated by the classical Hubel and Wiesel feedforward model due to their lack of a gain control mechanism, elicit responses once inhibitory feedback is introduced. Inhibitory connections within orientation columns increase the sharpness of orientation tuning. In addition, our simulation produces intracellular responses in which EPSP's are maximally tuned to the optimal orientation (Ferster 1986). We will show a video of these salient responses in our computer simulation.

362.8

INHIBITORY AND EXCITATORY CONTRIBUTIONS TO ORIENTATION TUNING IN THE CAT'S STRIATE CORTEX. F. Wörgötter* and U.Th. Eysel, Institute of Physiology, Ruhr-Universität Bochum, D-4630 Bochum, F.R.G.

Orientation specificity in the visual cortex can be generated by excitatory convergence of subcortical elements, intracortical excitation and intracortical inhibition (Ferster, D. & Koch, C., TINS, 10:487,1987). Our study utilized local lateral inactivation of cortical tissue by GABA microiontophoresis to investigate the influence of intracortical mechanisms. Recordings were made from cells in layers III-VI near area centralis in the striate cortex of anesthetized cats while stimulating with moving light bars. GABA microiontophoresis was performed at different distances relative to the recorded cell and changes of the response characteristics were quantified. Orientation tuning was significantly reduced for S- and C-cells during inactivation of lateral regions. Lateral inhibition contributing to orientation selectivity was demonstrated predominantly by local inactivation at distances of about 0.5 mm. Such loss of lateral inhibition in layer IV S-cells clearly shows that intracortical inhibition is involved in the generation of orientation tuning in addition to the direct CGL input. Lateral excitatory effects were most frequently affected by inactivation at distances of about 1.0 mm. To determine the interdependence of direction and orientation tuning correlation coefficients between the strength of both specificities were calculated and only a weak coupling was found. This supports our previous evidence that different mechanisms underly direction and orientation specificity (Eysel et al., J.Physiol, 399:657, 1988). The results further suggest that both excitatory and inhibitory connections from laterally displaced cells can contribute to the generation of orientation specificity.

362.9

SIMPLE CELL BEHAVIOR IS PREDICTED BY A PUSH-PULL MODEL INCORPORATING COSPATIAL LATERAL GENICULATE (LGN) CELL INPUT. S.S. Raab*, J. McLean*, R.A. Stepnoski*, L.A. Palmer. Dept. of Anatomy, Univ. of Penn., Phila., PA 19104.

We have developed a model of simple cell receptive fields (SRF) based on the following assumptions: linearity of summation in 2D space and time, biphasic time course of rectified LGN inputs and a Hubel-Wiesel geometry of LGN inputs configured as push-pull (class B) amplifiers. By push-pull, we mean that all points in the SRF are excited by inputs of one center sign (push) and inhibited by inputs of the opposite center sign (pull). We will show that this model accounts for the following aspects of SRF function: a) structure in space-space-time b) orientation selectivity c) spatial summation d) spatial frequency selectivity e) cospatial distribution of epsps to bright (dark) and ipsps to dark (bright) stimuli f) optimal direction and velocity g) action of on-channel blockade by retinal APB and h) action of GABA blockers.

362.10

MEASUREMENT OF A SIMPLE-CELL THRESHOLD FUNCTION IN CAT'S STRIATE CORTEX. *R.C. Emerson, M.J. Korenberg*, and M.C. Citron.* Ophthalmology and Ctr. for Visual Sci., U. Rochester, Rochester, NY 14627; Electrical Engrg., Queen's U., Kingston, Ontario, Canada, K7L 3N6; and Childrens Hosp. of LA, Neurology Research, P.O. Box 54700, Los Angeles, CA 90054.

We estimated the shape of a cortical threshold curve to gain insight into the operations that such a function could perform. We measured responses in an OFF area of a simple cell that showed strong threshold nonlinearities when it was presented with an optimally oriented bar whose luminance was modulated randomly. We then characterized the dynamic behavior of the pathway from retina to cortex by calculating a family of functions that are related to Wiener kernels. These functions are used to describe linear contributions to the neural response, and also nonlinear contributions that arise from interactions in time and intensity. Because cortical transformations are difficult to measure directly, we modeled the neural pathway as a cascade of a dynamic linear, a static nonlinear, and a second dynamic linear transformation (LNL model) in which only the linear transformations showed temporal filtering. Bloch's law suggests that the first transformation should be a linear temporal filter. The unique dependence of responses on the sequence of transformations in a system with alternating dynamic-linear and static-nonlinear stages facilitates testing model hypotheses about the sequence and nature of neural events. The resolution and completeness of the measurements allowed us to identify the first linear filter as lowpass and the second as more strongly bandpass. A fourth-degree polynomial representation of the intervening static nonlinearity, which we believe to represent the threshold nonlinearity of the measured simple cell, suggests that the onset of action-potential generation is gradual. This gradual transformation is well represented by a squaring function for positive input signals, i.e., a "half-squarer," which implies that a low order of nonlinearities will suffice for an accurate representation.

Our results suggest that (1) intracellulary, simple cells carry a highly linear representation of image luminance; (2) cortical thresholds are gradual, which makes them ideal for nonlinear operations such as squaring, an operation that has been proposed for movement energy models; and (3) the action-potential generator itself may initiate the mildly periodic process that we measure as the second dynamic linear filter. (Supported by EY06679, EY01319, NSERC (Canada), EY04711, and EY00250.)

362.11

X- AND Y-LIKE RECEPTIVE FIELD PROPERTIES IN CAT AREAS 17 AND 18. B. Jagadeesh* and D. Ferster. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

In a recent abstract, Ferster (Neurosci. Abstr., **13**:1449) reported that X- and Y-mediated synaptic potentials, identified by their thresholds to stimulation of the optic nerves, are largely segregated between areas 17 and 18 of cat visual cortex. Does this apparent segregation give rise to differences in receptive fields of cells in the two areas? Movshon *et al.* (J. Physiol. **283**:101) found that preferred spatial frequencies in area 17 were on the average 3-fold higher than those in area 18; this parallels the difference in preferred spatial frequencies of X and Y cells. We report two further distinctive features of receptives fields in area 17 and 18.

1) Y-cells are identified by their phase-independent, frequency-doubled response to stationary contrast-modulated gratings, which is most evident at high spatial frequencies. Spitzer and Hochstein (J. Neurophysiol. **53**:1245) failed to find evidence for this type of nonlinear response in the cells of area 17. We have confirmed this result and report in addition that the Y-like nonlinearity is present in many cells of area 18. 2) Y-cells have on average a 3-fold higher contrast sensitivity than X-cells (Troy, Vision Res. **27**:1733). Paralleling this difference, field potentials evoked in area 18 by stationary contrast-modulated gratings have approximately 3-fold higher contrast sensitivity than field potentials recorded simultaneously in area 17.

These differences in receptive field properties support the apparent segregation of X and Y input into areas 17 and 18 found intracellularly. Since with four independent methods, evidence for Y input to area 17 cannot be demonstrated, we suggest that the Y input to area 17 differs fundamentally from that in area 18, or is minimal in its extent.

362.12

SPATIO-TEMPORAL DISTRIBUTION OF STIMULUS-SPECIFIC OSCILLATIONS IN THE CAT VISUAL CORTEX I: LOCAL INTERACTIONS. C.M. Gray*, P. König*, A. Engel* and W. Singer (SPON: J.E.Skinner). Max-Planck-Institute for Brain Research, D-6000 Frankfurt/M 71, F.R.G.

An optimal stimulus presented within the receptive field of neurons in the cat visual cortex evokes a correlated oscillation of both the multiunit activity (MUA) and the local field potential (LFP) at a frequency near 40 Hz (C.M.Gray, W.Singer, Neurosci Abs 404.3, 1987). This finding suggests that neuronal oscillations provide a mechanism by which local intracolumnar populations of neurons temporally coordinate their activity. To test this hypothesis we recorded both MUA and LFP responses simultaneously across columns within a cortical layer, and within all layers of the same column using 4-16 closely-spaced electrodes (150-400 µm separation) in areas 17 and 18 of the cat visual cortex. The results demonstrate 1) that oscillations of both the MUA and LFP can occur in precise synchrony throughout all layers of the cortex, as well as independently in upper and lower layers, and 2) that the active population of cells engaged in the synchronous oscillations can extend from 300-1500 µm. Sharp boundaries of synchronous oscillatory activity occur at the junctions of columns having orientation preferences which differ by 60-90 degrees. The results support the hypothesis that neuronal oscillations provide a mechanism by which local populations of neurons synchronize their activity in response to specific sensory stimuli.

362.13

SPATIO-TEMPORAL DISTRIBUTION OF STIMULUS-SPECIFIC OSCILLATIONS IN THE CAT VISUAL CORTEX II: GLOBAL INTERACTIONS. W.Singer, C.M.Gray*, A.Engel* and P.König* (SPON:L.Peichl). Max-Planck-Institute for Brain Research, D-6000 Frankfurt/M 71, F.R.G.

In the previous report local populations of neurons, within columnar boundaries, were shown to engage in stimulus-specific coherent oscillations throughout all cortical layers. This suggests that relations between common, but spatially separate, features in the visual field may be established by the synchronization of oscillatory responses in different parts of the visual cortex. To test this hypothesis we recorded multiunit activity (MUA) and local field potential (LFP) responses in area 17 of the cat from multiple electrodes separated by 2-8 mm in the cortex while stimulating the respective receptive fields simultaneously with moving light bars. Cross correlation and coherence analysis of the signals demonstrated 1) that significant stimulus-dependent correlations of oscillatory responses occur between columns within area 17 separated by 2-8 mm, 2) that the correlated oscillations occur in phase with a variation of phase lag in the range of ± 3 ms, and 3) that the probability for correlated activity is related to the stimulus selectivity of the respective recording sites. These data support the hypothesis that oscillatory responses in spatially separate regions of the cortex may transiently synchronize and thereby define relations between stimuli with common features.

363.1

MULTIPLE CALCIUM CURRENTS IN CULTURED EMBRYONIC AMPHIBIAN SPINAL NEURONS. M.E. Barish, Department of Physiology and Biophysics, University of California, Irvine, CA 92717.

As part of an analysis of the roles of ion channel activity in neural development, I have studied the Ca currents of cultured embryonic *Xenopus* neurons during the period of initial differentiation and neurite outgrowth.

Cultures are prepared from nerve and muscle precursor cells isolated from the spinal region of the neural plate and underlying mesoderm of late neural plate-stage embryos. Ca currents in differentiating neurons are studied using conventional whole-cell tight-seal techniques. Cells are grown on serum-coated glass coverslips to minimize neurite growth, and neurons with processes extending less than one soma diameter can be studied routinely. Ca currents show monotonic activation and rapid deactivation, indications of good spatial control of membrane voltage.

Three components of Ca current can be separated based on kinetics and pharmacology. A fast transient component shows an activation threshold of ~-40 mV, and activation and steady-state inactivation curves overlap between -40 and -30 mV. These transient currents can be blocked by 100-150 μM Ni. In the presence of Ni, currents showing incomplete slow inactivation (during 1 sec depolarizations) are recorded at voltages >-30 mV. The inactivating portion shows an activation threshold of ~-30 mV, and overlap of activation and inactivation curves (measured using 1 sec conditioning and test depolarizations) occurs between -30 and +10 mV. Shifting the holding voltage to -40 mV exposes a non-inactivating inward current that can be activated at voltages >-10 mV. These components correspond to the T-, N- and L-type Ca currents that have been described in other excitable cells.

The resting potential of these neurons is between -50 and -60 mV, and the action potential threshold is about -25 mV. Thus two of the components of Ca current observed may mediate Ca entry during subthreshold voltage excursions.

363.2

LOW-THRESHOLD Ca CHANNELS OF ISOLATED RAT HYPOTHALAMIC AND HIPPOCAMPAL NEURONS ARE DIHYDROPYRIDINE-SENSITIVE.
N. AKAIKE*, Y. OSIPCHUK*, K. MURASE, A. YOSHIDA* and Y. IKEMOTO* (SPON: R.SHINGAI). Dept. of Physiol., Fac. of Med., Kyushu Univ., Fukuoka, 812, Japan.

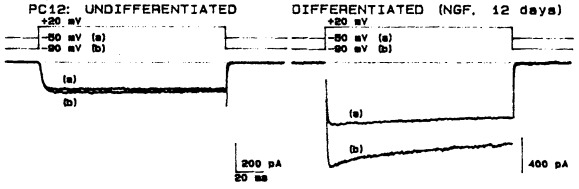
Electrical and pharmacological properties of low-threshold Ca channels have been studied in neurons isolated acutely from the hypothalamus and hippocampus of adult rats using both intracellular perfusion and concentration-clamp techniques.

In most cells a definite low-threshold inactivating component was found. The low-threshold component was activated at membrane depolarization to -60 mV from a holding potential level of -100 mV, and it inactivated exponentially in a potential dependent manner. The steady-state inactivation occurred at very negative membrane potentials, reaching 50% level at -90 mV. Low-threshold Ca channels were blocked in the order of La > Zn > Cd > Ni > Co and of flunarizine > nicardipine > nifedipine > nimodipine > D-600 > diltiazem. Substitution of Ba for Ca reduced the current by 30-50% while substitution of Sr for Ca did not produce definite changes in current amplitude.

363.3

EXPRESSION OF CA^{2+} CHANNELS IN DIFFERENTIATED PC12 CELLS. Mark R. Plummer, Diomedes E. Logothetis*, Peter Hess*. Dept. of Mol. and Cell. Physiology, Harvard Med. School, 25 Shattuck St., Boston, MA 02115.

Differentiation of PC12 cells induced by the presence of NGF for >10 days leads to a 5-fold increase in the density of Ba^{2+} current in the cell body and to a marked increase of the fraction of Ba^{2+} current which is irreversibly blocked by ω-Conotoxin. Holding potentials negative to -40 mV recruit a current component which inactivates slowly (τ=50-100 ms) and incompletely. The single channel activity underlying this component of whole cell current is similar in its main conductance (18-22pS), activation range, and mean open time to the dihydropyridine (DHP) sensitive L-type calcium channel, but it can be distinguished from the L-type channel by its lack of sensitivity to the DHP agonist (+) 202-791, inactivation rate during test depolarizations, and more negative steady-state inactivation range.



363.4

VOLTAGE-DEPENDENT CALCIUM CONDUCTANCES AND MAMMILLARY BODY NEURONS AUTORYTHMICITY: AN *IN VITRO* STUDY. A. Alonso* and R. Llinás. (SPON: D. Sanes). Dept. of Physiology. & Biophysics, NYU School of Medicine, New York, NY 10016.

Current and voltage-clamp recordings from medial mammillary and premammillary body neurons indicate the presence of three voltage-dependent Ca-conductances. In current-clamp studies, depolarization from a membrane potential of -80 mV, elicited typical low-threshold calcium spikes (LTS). These were often followed by prolonged plateau potentials (PPs), which were especially clear after TTX. Depolarizations from a more positive potential (-50 mV) could evoked PPs in isolation from the LTS, as the conductance responsible for the LTS was close to total inactivation at -50mV. After TEA, the PPs always generated high threshold Ca-spikes. Under voltage clamp from a holding potential of -85 mV a typical transient inward Ca-currents were first observed at -65 mV reaching a maximum peak at approximately -40 mV. A second inward Ca-current with slower activation and inactivaton kinetics and a higher activation voltage followed the transient ICa. Near -20 mV the voltage control was often lost as high threshold spikes were invariably evoked. These findings indicate that three Ca-conductances are present in mammillary neurons. These provide the electroresponsiveness needed to support the pacemaker authorhythmicity involved in the generation of theta rhythm in the limbic system. Supported by NIH grant NS13742 and a Fellowship to A.A. from theMinisterio de Educacion y Ciencia (Spain).

363.5

INACTIVATION OF Ca CURRENT IN ACUTELY DISSOCIATED HIPPOCAMPAL PYRAMIDAL CELLS

Alan R. Kay.* (SPON: D. Kleinfeld) Dept. Neurol., Columbia Univ. CPS.,NY,NY 10032 and AT&T Bell Labs., Murray Hill, NJ 07974.

The Ca^{2+}-current in acutely dissociated hippocampal CA1 pyramidal cells, activated rapidly (1-2 ms) on depolarizing the cell to potentials above -40 mV. The current then inactivated with a slow time course that was well characterized by the sum of 2 exponentials (time constants ~ 200 and 2000 ms) plus a constant offset. Both time constants decreased monotonically with increasing step potentials, suggesting a voltage dependent mechanism for inactivation. However, when a protocol to disclose Ca^{2+}-dependent inactivation was used (an inactivating prepulse (7s) to varying potentials, followed by a step to a constant potential to assay the amount of remaining current) the magnitude of all three components in the postpulse exhibited a minimum at the peak of the IV curve. The resulting U-shaped dependence of postpulse amplitude on voltage suggests Ca^{2+}-induced inactivation, however, contrary to the expectations of this form of inactivation, when the fast Ca^{2+}-buffer BAPTA, was included in the intracellular medium and Ba^{2+} extracellularly, the U-shape of the inactivation curve persisted. Moreover, under conditions where all current passing through the Ca^{2+} current was borne by Na+,the U-shape of the inactivation curve still persisted.
Supported by NIH grant NS 24519 to R.K.S. Wong, Columbia University.

363.6

RELATIONSHIP BETWEEN CALCIUM CURRENTS, I_{Ca}, AND INTRACELLULAR CALCIUM CONCENTRATION, $[Ca^{2+}]_i$, IN SENSORY NEURONS. Stanley A. Thayer* and Richard J. Miller Dept. of Pharm. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637

Simultaneous whole cell patch clamp and fura-2 microfluorimetric recordings of I_{Ca} and $[Ca^{2+}]_i$ were made from sensory neurons grown in primary culture from the dorsal root ganglion of the rat. Cells were held at -80 mV and depolarized to 0 mV eliciting a I_{Ca} that resulted in a $[Ca^{2+}]_i$ transient which lasted long after the cell had repolarized and the current stopped. When the cell was depolarized for different test pulse durations the relation between the integrated I_{Ca} and the peak of the $[Ca^{2+}]_i$ transient saturated at long test pulse durations. Thus, there appears to be a ceiling that limits the maximum obtainable $[Ca^{2+}]_i$ resulting from Ca^{2+} influx via Ca^{2+} channels. Higher $[Ca^{2+}]_i$'s were detected when, for example, Ca^{2+} flowed into the cell from a loss of seal resistance. Calculations of the integrated I_{Ca} and the peak $[Ca^{2+}]_i$ for currents and transients elicited at different test potentials also yielded integrated I_{Ca} versus peak $[Ca^{2+}]_i$ transient curves which saturated. When neuropeptide Y, which acts via its receptor and a G protein to inhibit I_{Ca}, was applied to a cell depolarized for 320 ms at 20s intervals a marked inhibition of I_{Ca} was observed with no significant effect on the peak of the $[Ca^{2+}]_i$ transient. However, when the test pulse was shortened to 80 ms a similar inhibition of I_{Ca} resulted in a significant inhibition of the $[Ca^{2+}]_i$ transient as well. Thus, inhibition of I_{Ca} was reflected by a change in the peak $[Ca^{2+}]_i$ only when submaximal $[Ca^{2+}]_i$'s were reached, implying that modulation of $[Ca^{2+}]_i$ is dependent on the activation state of the cell.

363.7

Modulation of calcium currents by Neuropeptide Y in rat myenteric neuron cultures. Lane D. Hirning*, Aaron P. Fox*, & Richard J. Miller (SPON: M.C. Nowycky), Dept. of Pharmacological and Physiological Sciences, The University of Chicago, 947 E. 58th St., Chicago, Il. 60637.

Our previous studies showed that Neuropeptide Y (NPY), acting via a GTP binding protein, inhibited primarily the inactivating component of whole-cell calcium current. Cell-attached patch recordings, with 90 mM Ba^{2+} in the patch pipette, demonstrated the presence of two types of unitary events with different amplitudes. The smaller amplitude Ca channel ("N-type"), 14 pS single-channel slope conductance, exhibited unitary events unaffected by the dihydropyridine Ca channel agonist Bay K 8644. Holding potentials more negative than -40 mV were required to reprime most of the N-type channel activity, although some openings were apparent even after extended periods at HP = -40 mV. The second type of Ca channel ("L-type"), 27 pS slope conductance, had open times that were dramatically lengthened by Bay K 8644. Furthermore, the L-type openings were relatively insensitive to the holding potential; the channels were largely available at HP = -40 mV. In 5 large multi-channel patches, the addition of 100 nM NPY outside of the patch pipette had no effect on the Ca channel currents under the patch pipette, implying that a readily diffusible second messenger was not involved. 100 nM NPY dramatically inhibited whole-cell currents and the depolarization induced calcium influx measured by Fura-2. These preliminary data suggest that NPY acts via a GTP-binding protein that may couple directly to N-type Ca channels.

363.8

ω-CONOTOXINS AND TWO CLASSES OF VOLTAGE-SENSITIVE CALCIUM CHANNEL TARGETS. L.J. Cruz, J.S. Imperial*, G.C. Zafaralla*, J. Haack and B. M. Olivera. Dept. of Biology, University of Utah, Salt Lake City, Utah 84112 and Dept. of Biochemistry & Marine Sci. Inst., Univ. of the Philippines, Metro Manila.

The ω-conotoxins are peptide toxins which inhibit voltage-sensitive Ca channels; at least two classes of ω-conotoxin targets can be distinguished. Not all Ca channels at vertebrate neuromuscular junctions are inhibited by ω-conotoxins; ω-conotoxin GVIA blocks avian, but not murine neuromuscular junctions. However, i.c. injection of mice causes a characteristic shaker syndrome, suggesting a class of ω-conotoxin sites in the CNS that differ from neuromuscular presynaptic targets. Biochemical evidence is also consistent with two classes of ω-conotoxin targets, one with a subunit MW of 135-160 K (efficiently crosslinked using disuccinimidyl suberate), and a second class with significantly higher molecular weights (200-300 K), crosslinked with photoactivatable ω-conotoxin GVIA derivatives. Crosslinking the 135-160 K target with ω-conotoxin is correlated to neuromuscular junction block. Using ω-conotoxin affinity chromatography, the two classes can be separated. ω-conotoxins from different Conus venoms often differ in their receptor subtype specificity. We have purified and characterized three new ω-conotoxins, SVIA, SVIB and TVIA. The receptor subtype specificities of the new ω-toxins are presently being defined. (Supported by GM22737).

363.9

TRANSIENT INHIBITION OF CALCIUM CURRENT BY ω-CONOTOXIN (ω-CgTX VIA) IN RAT RETINAL GANGLION CELLS. Andreas Karschin* and Stuart A. Lipton (SPON: H. Wässle). Max-Planck Inst. für Hirnforschung, D-6000 Frankfurt, FRG, and Dept. of Neurology, Children's Hospital & Harvard Medical School, Boston, MA 02115.

Calcium currents were recorded with patch electrodes in both the whole-cell and single-channel (cell attached) configurations. Isolation of currents was achieved as described by Lipton & Tauck [*J. Physiol.* 1987;385:361]. During whole-cell recording, application of voltage protocols [Fox, Nowycky & Tsien, *J. Physiol.* 1987;394:149] suggested the presence of multiple components of calcium current. Test potentials to -40 mV from a holding potential of -90 mV activated a transient, rapidly inactivating current (τ = 10-30 ms). Stronger depolarizations (to >-20 mV) activated a more slowly decaying component (τ = 100-200 ms) as well as a substantial steady-state level; these findings persisted when the extracellular solution was changed from 10 mM Ca^{2+} to Ba^{2+}. Some cells displayed only one or two of these components (e.g., transient and sustained) while others had all three. The transient (T-type) current was nearly unchanged when equimolar Ba^{2+} was substituted for Ca^{2+}, whereas the other component(s) increased. Dihydropyridines yielded the expected results on the sustained (L-type) component. However, unlike the experience in DRG neurons [McCleskey et al., *PNAS* 1987;84:4327], 10 μM ω-CgTX VIA transiently and reversibly suppressed all components of calcium current.

363.10

CELLULAR DISTRIBUTION AND LATERAL MOBILITY OF A NEURONAL CALCIUM CHANNEL VISUALIZED WITH FLUORESCENT ANALOGS OF ω-CONOTOXIN. Owen T. Jones*, Diana L. Kunze and Kimon J. Angelides. (SPON:J.Dani). Dept. Physiol. & Mol. Biophys., Baylor College of Medicine, Houston, Texas 77030.

To determine the topography and mechanisms that regulate neuronal Ca^{2+} channel distribution during development and synaptogenesis, biologically active fluorescent analogs of ω-conotoxin (a high affinity probe for neuronal Ca^{2+} channels) were prepared. Electrophysiology and binding studies show that these analogs retain their high affinity for the Ca^{2+} channel (Kd=6nM).

Digital fluorescence imaging of rat hippocampal CA1 neurons labeled with fluorescent conotoxin analogs reveal a non-uniform distribution of the channels. The fluorescence was most intense on the cell body and was clustered in hotspots that coincided with axo-somatic synapses. Hotspots were also seen on processes and on cell bodies with no discernible synaptic contacts. Measurement of the lateral mobility of the channels on the cell body by fluorescence photobleach recovery shows that 70% are immobilized, the remaining fraction move at a rate of 5 X 10^{-10} cm^2/s. In contrast, on the cell body, Na^+ channels are diffusely distributed and rapidly mobile (10^{-9} cm^2/s). Thus, there appears to be specific mechanisms that regulate Ca^{2+} channel distribution and organize and immobilize these channels into discrete regions of the pre- and postsynaptic membranes.

363.11

EFFECTS OF Ca-CHANNEL AGONISTS AND ANTAGONISTS ON THE SPECIFIC BINDING OF [^{3}H]-NITRENDIPINE (NP) TO CANINE CEREBRAL CORTEX SYNAPTIC MEMBRANE (SPM) AND POSTSYNAPTIC DENSITY (PSD) FRACTIONS. P. Siekevitz and M. LeDoux*, Lab. Cell Biol., Rockefeller Univ., New York, NY 10021

The specific binding of the antagonist NP ($2x10^{-9}$M) to both SPM and PSD fractions was inhibited in a linear fashion by nifedipine (NF) antagonist, by BAY K8644 agonist, by Sandoz Lts. enantiomers, +(202-791) agonist and (-) 202-791 antagonist, reaching in all cases 100% inhibition at 10^{-6}M. Each of the (+) and (-) enantiomers caused a three-fold increase in the K_D for NP binding, but also decreased somewhat the Bmax. At $8x10^{-8}$M all four compounds inhibited the binding by ~50%. However, when $8x10^{-8}$M NF and $8x10^{-8}$M BAY K8644 were added together, the resultant inhibition was additive, reaching 80-100%, with both fractions. The same result obtained when the (+) and (-) enantiomers were added together each at $8x10^{-8}$M. A similar result was obtained when the ratio of NP receptors to NP conc. was changed. The binding of NP to PSDs was reversible. Thus, there occured a 25% loss of PSD-bound NP after incubation at 25°C for 40 min. Each of the enantiomers, at $8x10^{-8}$M, increased the loss to 45-50%, but when added together, the loss was additive, 100%. A possible explanation is that the binding of NP is competed for at the same site by other antagonists, such as NF and (-)202-791, and that the agonists, BAY K8644 and (+)202-791 bind to another site which allosterically interacts with the antagonist site, reducing NP binding.

363.12

ISOLATION OF A VOLTAGE-DEPENDENT CALCIUM CHANNEL FROM MAMMALIAN CNS. (SPON J. Ransohoff) B. Cherksey, M. Sugimori, B. Rudy and R.Llinás. Dept. Physiol. & Biophys., NYU Med. Ctr. New York, N.Y. 10016

A calcium channel blocking factor purified from *Agelenopsis aperta* venom was utilized to isolate voltage-dependent ion channels from central neurons. An affinity gel constructed with this factor allowed the extraction of protein from guinea pig solubilized whole brain homogenate. The functional activity of the resulting protein solution was assessed using the lipid bilayer technique. The electrical activity was measured in solutions containing: 80 mM $BaCl_2$, 10 mM HEPES pH 7.4 on the *trans* side; 150 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES pH 7.4 on the *cis* side (side of potential application and vesicle addition). The most typical channel observed was characterized by a slope conductance of 15-20 pS. The I-V curves exhibited some rectification. E_{rev} was at +90- +120 mV. The channel had a mean open-probability of < 7% at -70 mV and opened with increasing frequency as the potential was made more positive. Single-channels were blocked by *trans* addition of Cd or Co at concentrations below 100 μM and by the active factor. With $MgCl_2$ absent from the *cis* solution, monovalent ions could traverse the channel. For 150 mM concentration of monovalent ions a conductance of 100 pS was determined for K^+ and 20 pS for Cs^+.

We conclude that purified factor has allowed us to isolate a CNS voltage-dependent calcium channel with properties similar to those underlying the high- threshold calcium spikes in Purkinje cells, which are also blocked by a toxin preparation derived from this venom (Sugimori&Llinás 1987 Neuroscience Abst.) Supported by NIH NS13742.

364.1

EMBRYONIC CHICK MOTONEURONS RESPOND TO NGF IN VITRO AND RETROGRADELY TRANSPORT NGF IN VIVO. D.B. Wayne* and M.B. Heaton. Dept. of Neuroscience, University of Florida, Gainesville, FL, 32610.

The role of NGF in stimulating outgrowth from neurons of the PNS has long been recognized. Here we present evidence that embryonic chick motoneurons of the brainstem and spinal cord are NGF-responsive. Brainstem trigeminal and spinal cord lumbosacral motoneurons specifically transport ^{125}I-NGF transiently early in development and respond to NGF in vitro during that time.

Trigeminal motoneurons were found to specifically transport ^{125}I-NGF following target (jaw) injections at 4 1/2 and 5 but not at 10 days of incubation. Neurons of the spinal lateral motor column (LMC) were found to specifically transport ^{125}I-NGF following target (limb) injections at 5 and 6 but not at 13 days of incubation.

Dissociates of the trigeminal basal plate (VBP) (day 4) and the ventral spinal cord (SCBP) (day 5) were cultured in the presence of NGF or in control medium. NGF did not affect survival of these populations, however, process outgrowth was enhanced. NGF significantly increased the neurite quantity of SCBP dissociates at 24 and 48 hours while neurite initiation was significantly increased in VBP dissociates after 48 hours.
Supported by NIH grant NS-20387.

364.2

STRUCTURE AND DEVELOPMENTAL EXPRESSION OF THE NGF RECEPTOR IN CHICK BRAIN . T.H. Large, G. Weskamp* and L.F. Reichardt. Howard Hughes Medical Institute and Dept. of Physiology, Univ. of California, San Francisco, CA 94143

We have determined the nucleic acid sequence of the nerve growth factor receptor from chicken. A cDNA probe containing the coding region of the low affinity (fast) NGF receptor from rat was used to isolate a 3.7 kb insert from an E13 chick brain cDNA library. Southern blots of chick genomic DNA probed with the chick cDNA or rat NGF receptor cDNA showed similar banding patterns, strongly suggesting the chick clone encodes the low affinity NGF receptor. In support of this, the deduced amino acid sequence for the chick receptor shows 70% sequence identity with the rat and human receptors. Of the two in-frame ATG sequences found upstream of the signal peptide in rat and human, only the second is conserved in chick, suggesting this site may be favored for initiation of translation. In the extracellular domain, the four cysteine-rich repeating elements show 75% sequence identity, including conservation of all 24 cysteine residues. The region surrounding the membrane-spanning domain is the most highly conserved part of the receptor (95% identical), suggesting this region may be critically important for receptor function. Within the intracellular domain (60% identical), the C-terminus is the most strongly conserved (80% identical). Northern blot analysis indicated a receptor mRNA of approximately 4.0 kb is expressed in E13 sympathetic ganglia but not adult liver. Receptor mRNA also is relatively abundant in retina, optic tectum, cortex, cerebellum and brainstem from E13 chicks, but is barely detectable in adult brain regions. Thus, the NGF receptor appears to be transiently expressed throughout the chick brain during embryonic development.

364.3

SUBSTANCE P MODULATES RELEASE OF LOCALLY SYNTHESIZED NERVE GROWTH FACTOR FROM RAT SAPHENOUS NERVE NEUROMA

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The most proximal segment of the transected saphenous nerve, a neuroma-like structure, was used as a model to study mechanisms in nerve growth factor (NGF) synthesis and release. In saphenous nerve neuromata of adult rats a long-term increase in NGF protein was detected by an enzyme-linked immunoassay after nerve transection. There was a rapid 3 fold increase in NGF levels 12 h after injury, which reached peak values (9 fold) after 4 days and subsequently fell to 2 fold elevated levels within 3 weeks. Quantitative Northern blots showed, that NGF mRNA levels increased rapidly in neuroma tissue, reaching a maximum about 2-4 days after transection indicating that the increase in NGF in response to injury is due to local biosynthesis.

Superfusion of the neuroma in situ revealed a continous basal release of NGF protein (2pg/min) for at least 45 min, which was drastically decreased by substance P (SP) in a dose-dependent manner. Maximum inhibition (85 ± 10%, N=8) occurred at a concentration of 0.1 µM. Neurokinin A was less potent than SP. Neurokinin B, as well as other peptides such as calcitonin gene-related peptide, somatostatin and neurotensin at concentrations up to 50 µM did not significantly affect NGF release.

These results suggest that SP may specifically modulate the availability of NGF in the microenvironment of regenerating nerve fiber endings.

364.4

THE EXPRESSION OF MESSENGER RNAS ENCODING NERVE GROWTH FACTOR AND ITS RECEPTOR IN THE EMBRYONIC HIPPOCAMPUS. **B. Lu, C. R. Buck, C. F. Dreyfus, and I. B. Black. Div. of Developmental Neurology, Cornell Univ. Med. Col., New York, N.Y., 10021**

Increasing evidence suggests that Nerve Growth Factor (NGF) plays a role in the ontogeny of the basal forebrain-hippocampal system. However, it is not known whether NGF regulates the development of the hippocampus (Hi) itself. While high levels of NGF protein in embryonic Hi have been detected and reported, RNA blot hybridization analysis failed to identify NGF message. We have employed sensitive nuclease protection assays to evaluate levels of NGF and NGF receptor (NGF-R) messages in the fetal Hi.

Using a sensitive ribonuclease protection assay, we found that NGF mRNA is expressed as early as embryonic day 16, and the message increases progressively throughout the postnatal period. Further, S1 nuclease experiments revealed that NGF-R message is also expressed in embryonic Hi. However, the receptor message declines postnatally. Thus, both NGF and NGF-R are locally synthesized in the embryonic Hi. These data suggest that NGF may play a role in the developing embryonic Hi. We are currently defining the specific cell types that express NGF and NGF-R, and studying the precise role of NGF in Hi ontogeny. (supported by NIH grant HD23315 and a grant from the Alzheimer's Disease and Related Disorders Assoc.)

364.5

LOCALIZATION OF HIGH AFFINITY NGF RECEPTORS TO GABAERGIC NEURONS IN THE CULTURED BASAL FOREBRAIN (BF). C.F. DREYFUS, P. BERND AND I.B. BLACK, Div. Devel. Neur., Cornell Med. Coll., New York, N.Y. 10021 and Dept. Anat. and Cell Biol., SUNY Health Science Center, Bklyn, N.Y. 11203.

Previous studies indicate that nerve growth factor (NGF) specifically elevates choline acetyltransferase (CAT) in BF cultures. To identify mediating mechanisms, we employed simultaneous radioautography, visualizing putative high-affinity ^{125}I-NGF receptors (NGF-R), and immunocytochemistry, detecting CAT+ cells. High-affinity NGF-R's were detected on CAT+ cells, suggesting that NGF directly regulates cholinergic neurons by binding to high-affinity receptors. Unexpectedly, we also observed a population of noncholinergic, NGF-R+ cells.

To further identify these potentially NGF receptive cells, we now have examined BF dissociates for co-localization of high-affinity NGF-R's and other transmitter traits. Gamma aminobutyric acid (GABA) cells were identified immunocytochemically using two polyclonal anitsera, one produced against a glutaraldehyde conjugate of GABA (Chemicon International, Inc.) and a second raised against GABA conjugated to bovine serum albumin (Incstar Corp.). In both cases, high-affinity NGF-R's were detected on GABA+ cells. However, only a subset of GABA+ cells exhibited NGF-R's, while other NGF-R+ cells were GABA negative. Our observations suggest that GABA+, as well as CAT+, cells express NGF-R's. Presently, we are further characterizing these diverse populations. (Supported by NS 20788, HD 23315 and the Alzheimer's Disease and Related Disorders Assoc., Inc.)

364.6

STUDY OF NGF RECEPTOR IN RAT POSTERIOR PITUITARY

Q. Yan, P. E. Lampe*, H. B. Clark and E. M. Johnson, Jr. Dept. of Pharmacology, Washington Univ. Med. Sch., St. Louis, MO 63110.

The expression of nerve growth factor (NGF) receptor was studied in rat pituitary gland by immunohistochemistry using 192-IgG, a specific anti-rat NGF receptor monoclonal antibody. The NGF receptor immunoreactivity (NGFRI) in pituitary gland started to appear diffusely at about 40 days of age. At 2-, 4- and 12-month-old, the staining was intense but became diffuse and less distinct in 17-month-old animals. The staining in pituitary was only seen in the posterior but not in the intermedial and anterior lobe. The staining formed patches in cross section and a bundle pattern in sagittal section of the posterior lobe. The staining was also seen in pituitary stalk and the outer layer of medial eminence but was not found in hypothalamus. The same staining pattern was seen in both sexes of 2-month-old rats and castration of males at 30 days did not affect the staining in the adults. ^{125}I-NGF crosslink/192-IgG immunoprecipitation followed by SDS-PAGE autoradiography, showed that the NGF receptor in pituitary gland had the same molecular weight (90 KD) as previously reported. The amount was much greater in the posterior lobe than in the anterior lobe. Preliminary double labeling results showed that the NGFRI elements in the posterior pituitary were neurophysin negative. The cellular characterization of NGFRI is now in progress by both immunofluorescent double staining and by immunoelectromicroscopy.

364.7

RETROGRADE TRANSPORT OF NERVE GROWTH FACTOR (NGF) FROM HIPPOCAMPUS TO SEPTUM IS IMPAIRED IN AGED RATS. Sookyong Koh and Rebekah Loy Dept. of Neurobiology & Anatomy, Univ. of Rochester Rochester, NY 14642

Previous work in our laboratory has demonstrated age-related loss of NGF sensitive neurons in rat basal forebrain (*Brain Research* 440). Decrease in the number of NGF receptor immunoreactive neurons appears to reflect a loss of receptor expression by a process of chronic cellular dysfunction and not primarily cell death. The present study was undertaken to determine whether this reduction in NGF receptor signifies an impairment of receptor-mediated axonal transport of NGF from target area to the cell bodies of NGF responsive neurons. Female Fisher 344 rats at 29 months of age (n=5) and 13 months of age (n=4) were used. I^{125} NGF was injected intrahippocampally into dentate gyrus/CA3 region and the brains taken out 24 hours later. Labeled neurons in the medial septal nucleus (MS) and vertical limb of diagonal band nucleus(VDB) were visualized autoradiographically. 15 coronal sections were selected from each brain and nucleus ipsilateral to the injection sites counted. Within the MS/VDB of the aged group, the mean number of radiolabeled neurons is reduced 30% compared to the young adult (255± 48 vs 369 ±39 ; $p \leq 0.005$). This loss of labeled neurons parallels reduction of 32% in the number of NGF immunoreactive neurons in aged rats previously documented. Loss of capacity of basal forebrain neurons to bind and transport NGF from their terminals in the hippocampus would result in decrease in NGF delivered to the cell bodies and may promote cellular dysfunction and death of neurons in aging. Supported by NRSA MH-09541 (SK) and ADRDA PRG-86-041(RL). Fisher 344 rats were provided by NIA for predoctoral studies (SK).

364.8

THE NERVE GROWTH FACTOR COMPLEX FROM *MASTOMYS NATALENSIS*: cDNA CLONING OF α- AND γ-LIKE MOLECULES. M. Fahnestock and R. A. Bell*. SRI International, Menlo Park, CA 94025.

Nerve growth factor (NGF) is isolated from the submaxillary glands of the mouse and of the African rat, *Mastomys natalensis*, as a high-molecular-weight complex. In the mouse, this complex consists of three subunits: βNGF, which has nerve-growth-promoting activity; the γ subunit, a member of the kallikrein family of serine proteases; and the α subunit, an inactive member of the kallikrein family. The lack of activity of the mouse α subunit may result from amino acid substitutions near the active site or from N-terminal changes that prevent zymogen activation.

The *Mastomys* NGF complex differs from the mouse complex in that in *Mastomys* the γ subunit is either missing or more loosely bound than in the mouse. We have previously isolated a cDNA clone coding for *Mastomys* βNGF whose sequence suggests reasons for the differences in subunit interactions between mouse and *Mastomys* complexes. Furthermore, like the mouse, *Mastomys* contains a large family of homologous kallikreins. We have isolated cDNA clones for a number of these kallikreins, one of which shares a high degree of sequence homology with the γ subunit of mouse 7S NGF. However, the clone contains an Arg→Leu substitution at residue -1 that might prevent zymogen activation in a manner similar to the mouse α subunit. The sequences of the isolated cDNAs provide information on conserved and variable regions of the *Mastomys* kallikreins and are important for further studies of the α and γ subunits of NGF.

364.9

EFFECTS OF NGF, CNTF, EGF, PDGF, TGF-β, INSULIN, AND RETINOIC ACID ON SEPTAL AND PEDUNCULOPONTINE CHOLINERGIC NEURONS IN CULTURE. B. Knusel* and F. Hefti (SPON: W. Sheremata), Dept. of Neurology, Univ. of Miami, Miami, FL 33101.

In order to study the trophic control of different populations of central cholinergic neurons, various known trophic factors were added to primary cultures of dissociated septal and pedunculopontine neurons of fetal rat brain. NGF, as shown previously, increased the activity of choline acetyltransferase (ChAT) in septal cultures severalfold as compared to controls. In contrast, ChAT activity of pedunculopontine cultures, which contain a second major population of centrally ascending cholinergic neurons, was not influenced by NGF. NGF did not affect protein levels in either type of culture. Retinoic acid has been reported to stimulate expression of NGF and NGF receptors in neuroblastoma cells but failed to elevate ChAT activity in both culture systems and did not affect the NGF mediated elevation of ChAT activity in septal neurons. TGFβ mildly stimulated ChAT activity in septal cultures. High concentrations of insulin increased ChAT activity in pedunculopontine and to a lesser extent in septal cultures. EGF and insulin elevated protein levels in both culture systems in a dose dependent manner. CNTF and PDGF failed to affect ChAT activity and protein levels in both septal and pedunculopontine cultures.

364.10

Interleukin-1 Regulates NGF mRNA in Rat Hippocampal Cultures. W.J. Friedman, L. Lärkfors*, T. Ebendal*, and H. Persson*, Lab. of Molecular Neurobiology, Karolinska Institute, Stockholm, Sweden, and Dept. of Developmental Biology, Uppsala University, Uppsala, Sweden

Abundant evidence indicates that nerve growth factor (NGF) is synthesized in rat hippocampus and cortex, and provides trophic support for basal forebrain cholinergic neurons. To investigate specific signals which regulate expression of this trophic factor we have examined dissociated cultures of rat hippocampus from embryonic day (E) 21.

Previous work has demonstrated increases in NGF mRNA and protein following lesions of the septo-hippocampal pathway. To determine possible underlying mechanisms, the effect of interleukin-1 on NGF in hippocampal cultures was examined. NGF mRNA was analyzed using Northern blots, and protein was detected using a highly sensitive enzyme immunoassay. Dissociates were grown at a density of one million cells per 35 mm plate for four days, at which time they were exposed to human recombinant interleukin-1 (IL-1) (10 U/ ml) for four hours. This treatment elicited a significant increase in NGF mRNA. Since IL-1 has been detected in the brain following injury, it may play a role in mediating the lesion-induced increase in NGF. Studies are now in progress to further characterize this effect.

364.11

ELECTRON MICROSCOPY OF NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY IN CEREBELLAR PURKINJE CELLS OF ADULT RATS PRETREATED WITH COLCHICINE. **E.P. Pioro, A. Ribeiro-da-Silva and A.C. Cuello. Dept. of Pharmacology and Therapeutics, McGill University, Montreal, PQ, H3G 1Y6.**

Nerve growth factor receptor (NGFr) immunoreactivity (IR), as revealed by the monoclonal antibody 192-IgG (Chandler, C. *et al. J Biol Chem 259*: 6882, 1984), is present in the cerebellum of postnatal rat (Eckenstein, F., *Brain Res 446*: 149, 1988). Its apparent immunocytochemical disappearance from adult cerebellum, however, does not correspond with relatively high levels of receptor IR detected here by radioimmunoassay (Taniuchi, M. *et al. PNAS USA 83*: 1950, 1986). We have shown that pretreatment of adult rats with colchicine allows visualization of NGFr-IR in Purkinje cells (Pioro and Cuello, *Brain Res*, in press). Here we report the ultrastructural appearance of such IR in these cells of Wistar rats which had received colchicine (27.5 mg/100 g) intracerebroventricularly 40 hours prior to aldehyde perfusion and tissue processing for electron microscopic (EM) analysis. The majority of peroxidase IR was localized to the Purkinje cell membrane although NGFr reaction product was also related to Golgi apparatus, rough endoplasmic reticulum and secondary lysosomes. Rarely, immunoreactive clathrin-coated vesicles (80-90 nm dia.) were seen near the cell membrane. This is the first EM demonstration of NGFr-IR in neurons of the central nervous system with evidence of NGFr synthesis, membrane-incorporation, internalization and degradation. Supported by the Medical Research Council (Canada).

364.12

NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY PRESENT IN AXONS AND EPITHELIAL CELLS OF THE RAT SKIN.

A. Ribeiro-da-Silva*, R.L. Kenigsberg* and A. Claudio Cuello.
Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1Y6.

The distribution of nerve growth factor receptor (NGFr) was investigated immunocytochemically in the skin of the lower lip of the rat. Tissue was fixed by vascular perfusion and then processed for light or electron microscopy. NGFr immunoreactive sites were revealed with the use of IgG 192 monoclonal antibody followed by a monoclonal anti-mouse IgG/anti-horseradish peroxidase bi-specific secondary antibody. NGFr immunoreactivity was found to occur in a patchy pattern in the plasma membranes of groups of basal keratinocytes and Merkel cells of the epidermis and outer root sheaths of hair follicles. Intense immunostaining was also present in the perineural cells and in axons of small cutaneous nerves. Some of these nerves were in close contact with the immunoreactive epithelial cells. Isolated immunoreactive nerve fibers could also be seen penetrating the epidermis while other immunostained nerves were surrounding the wall of blood vessels and glands. The physiological significance of these findings will be discussed.

364.13

RABBIT ANTI-NGF IgG REDUCES CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE HIPPOCAMPUS AND SEPTAL AREA OF NEONATAL RATS. G. Vantini, N. Schiavo*, M. Fabris*, L. Callegaro*, A. Di Martino*, P. Polato*, D. Guidolin* and A. Leon. Fidia Research Laboratories, Abano Terme, Italy.

Several recent observations have indirectly led to the suggestion that NGF may have a physiological role for cholinergic neurons of the basal forebrain. We here report that rabbit anti-NGF IgG decreases choline acetyltransferase (Chat) activity in the septum and hippocampus of newborn rats. In particular, the anti-NGF antibodies, intracerebrally injected (6 μg in 10 μl of phosphate buffered saline) on postnatal days (P) 2, 4, 6 and 8, produced on P9 a significant decrease (approx. 30%) of Chat activity. This decrease was less evident at P15 (15% decrease), while no significant changes were detected at P25. Furthermore, a marked reduction of Chat-positive neurons was observed in the septal area of 9-day-old rats treated with anti-NGF IgG. At all times, acetylcholine esterase activity remained unmodified.

These results provide a direct demonstration that endogenous NGF affects the function of forebrain cholinergic neurons. Thus, a lack of endogenous NGF or a reduced sensitivity to NGF may well underlie alterations and/or loss of these neurons known to occur in some neurodegenerative diseases.

TRANSMITTERS: ACETYLCHOLINE

365.1

POSTJUNCTIONAL MONITORING OF ACETYLCHOLINE RELEASE. R.J. Storella, M. Alexander[a] and S.A. Slomowitz[a]. Department of Anesthesiology, Hahnemann University, Philadelphia, PA 19102

Postjunctional monitoring is often used to assess changes in acetylcholine (ACh) release. Thus, the frequency-dependent decrease of either tension or endplate potentials during neuromuscular block suggests a decrease in ACh release. However, we hypothesized that changes in ACh release in the presence of α-bungarotoxin (αBT) would be masked from detection due to αBT's irreversible binding to ACh receceptors. The mouse phrenic-nerve diaphragm was maintained in a modified Krebs solution, aerated with O_2/CO_2 (95%/5%) at 37°C. The nerve was stimulated supramaximally and isometric tension recorded. Preparations were 70-80% blocked (0.2 Hz) with either d-tubocurarine (dTC) or αBT and tested with conditions assumed to either increase or decrease junctional ACh levels. Following either tetanus (200 Hz, 20 sec) or edrophonium (12.4 µM), dTC block was reversed. Also, $MgCl_2$ (0.3 mM) increased dTC block. In contrast, these same conditions had minimal effects on αBT block. Thus, changes in ACh release and duration during αBT block were not detected. Therefore, the minimal frequency-dependence of αBT block (observed at 2.0 Hz) may be due to an inability to detect changes in ACh release rather than an inability of αBT to cause such changes. Postjunctional monitoring of ACh release must be interpreted cautiously.

365.2

ACETYLCHOLINE ELICITS TWO TYPES OF RESPONSE IN LARGE CELLS OF BASAL FOREBRAIN CULTURES. M. Anderson, A.A. Khan and R.W. Baughman. Dept. of Neurobiology, Harvard Med. School, Boston, MA 02115.

The basal forebrain projects widely to neocortex and hippocampus, and receives cholinergic input from the pontine tegmentum. We explored the effects of acetylcholine on large neurons (20 - 30 um) of the basal forebrain in tissue cultures prepared from 7 - 10 day old Long-Evans rat pups. Neurons were enzymatically dissociated (Heuttner and Baughman '86) and plated in Eagles MEM containing 5% rat serum and 1 ug/ml 7S nerve growth factor. Whole cell patch electrodes of 4.5 - 14.5 Mohms resistance were used to record intracellularly from cells in 21 - 38 day old cultures. The cells had resting potentials of 61.8 $\pm$ 6.2 mV (n = 19) and input resistances of 243.2 $\pm$ 109.6 (n = 19). The effects of acetylcholine (ACh) were tested in cultures perfused with Hepes-buffered recording medium containing 1 uM tetrodotoxin. Puffer pipets (3 um diameter) were used to deliver 0.2 - 0.4 s pulses of recording medium alone, 10 uM ACh, 100 uM ACh, or a mixture of 10 uM ACh and 1 uM atropine. In some experiments, the bath was also perfused with 1 uM atropine. In different cells, ACh elicited either depolarizations or hyperpolarizations. In some cells, ACh elicited depolarizations up to 28 mV in amplitude with times to peak of 100 - 300 ms; the depolarizations were accompanied by a decrease in input resistance, and they were diminished in a partially reversible manner when the bath was perfused with 1 uM atropine. Pulses of control medium produced no change in membrane potential or input resistance. In other cells, ACh elicited long duration (2 - 4 s) hyperpolarizations up to 4.4 mV; the hyperpolarizations were accompanied by an increase in membrane resistance. Addition of atropine to the puffer solutions reversibly reduced the hyperpolarizations. These results suggest that ACh acts at muscarinic receptors on basal forebrain cells to produce two types of response. Supported by NIH EY03502 and ADRDA II-86-099.

365.3

MUSCARINIC EXCITATION OF INHIBITORY CIRCUITS IN NEOCORTICAL SLICES. B.W. Strowbridge and G.M. Shepherd, Departments of Neuroscience and Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

Acetylcholine (ACh) excites both pyramidal cells and inhibitory interneurons in the guinea pig neocortex. We have used local microapplication of ACh to probe inhibitory local circuits, measured by their postsynaptic actions on pyramidal cells.

With the excitatory M-1 response suppressed by pirenzepine, pyramidal cells responded to ACh with hyperpolarization. With low concentrations of ACh (1-5 mM), the response had several components. A slow hyperpolarization lasted 1-3 seconds, associated with increased conductance; it reversed at 85-95 mv. Superimposed on this response were 1-3 mv fast unitary-like hyperpolarizations ranging from 30-60 ms in duration and reversing at potentials 10-15 mv more depolarized than the slow response. These fast events were depolarizing in neurons impaled with KCl electrodes, suggesting they were chloride mediated. Bicuculline resistant slow events were also observed.

Our results suggest that local ACh applications can evoke several types of inhibitory responses, whose properties resemble those of fast and slow IPSPs. These responses may be mediated by the same circuit, since repeated ACh applications often showed a fast event immediately preceding the slow responses. (Supported by NIH NS-07609 and the Office of Naval Research)

365.4

CALCITONIN-GENE-RELATED-PEPTIDE ENHANCES THE RATE OF DESENSITIZATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IN CULTURED MOUSE MUSCLE CELLS.

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CNRS UA041149, Laboratoire de Neurobiologie Moléculaire, Institut Pasteur, Paris, France.

Calcitonin-gene-related-peptide (CGRP) is a neuropeptide which coexists with acetylcholine (ACh) in spinal cord motoneurons. The effects of CGRP on the functional properties of the nicotinic acetylcholine receptor (AChR) are examined by electrophysiological methods. Using the whole-cell patch-clamp technique and a mouse cell line derived from soleus muscle, we find that CGRP produces a progressive and reversible enhancement of the rapid decay phase of AChR desensitization. Single channel data further show that CGRP decreases ACh-activated channel opening frequency. This decrease takes place when CGRP and acetylcholine are applied on different areas of the cell surface and is thus most likely mediated by a second messenger system. CGRP is also shown to increase cAMP synthesis in this cell line. The effects of CGRP on macroscopic ACh-activated currents are mimicked by external application of forskolin (10µM) or by internal perfusion of the cell with cAMP (1mM). In both these cases, further application of CGRP does not produce additional enhancement of AChR desensitization. These results suggest that, on mouse muscle cells, CGRP regulates AChR desensitization by a mechanism which could involve, at least in part, cAMP-dependent phosphorylation of the AChR.

365.5

HALF-LIFE OF PHOSPHATIDYLCHOLINE IN CHOLINERGIC AND NON-CHOLINERGIC NEURONS. W.D. Blaker and A.F. Dobrenski* VA-MD Regional College of Veterinary Medicine, Va Tech, Blacksburg,VA 24061

To initiate an in vivo study of the hypothesis that choline-containing lipids in cholinergic nerve endings can serve as a source of choline for acetylcholine synthesis, we compared the apparent half-lives of labelled, axonally transported choline phospholipids in a cholinergic vs. a non-cholinergic brain pathway. [^{3}H]-choline and [^{3}H]-mevalonate were stereotaxically injected into the rat medial septum (site of cholinergic neurons projecting to the hippocampus) or the hippocampus (site of non-cholinergic neurons projecting to the contralateral hippocampus). The labelling of specific lipids in the hippocampi (septal injections) or contralateral hippocampus (hippocampal injections) was followed 1-12 weeks post-injection. The choline labelled exclusively phosphatidylcholine and sphingomyelin. The mevalonate labelled almost exclusively cholesterol, which was used to correct for injection and sample handling variability. Cutting the projection fibers post-injection was used to confirm that the hippocampal labelling was due to axonal transport rather than injected label diffusion. The half-lives of neither choline phospholipid was significantly different in the two pathways.

365.6

PEPTIDERGIC AFFERENTS TO FOREBRAIN CHOLINERGIC NEURONS. L. Zaborszky and A. Braun*. Dept. of Otolaryngology, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

In order to determine whether peptidergic afferents innervate cholinergic forebrain neurons, sections from rat brains were processed for the simultaneous light microscopic visualization of peptidergic fibers/terminals and choline acetyltransferase (ChAT) containing neurons, using nickel enhanced DAB for peptidergic terminals and DAB for identification of ChAT containing neurons. Antibodies against the following peptides were used: neurotensin, somatostatin (14, 28), CCK, Met-enkephalin, CRF, Neuropeptide Y, beta-endorphin, alfa-MSH and CGRP. There is a differential but partially overlapping distribution of the various peptidergic fibers to the subdivisions of the cholinergic forebrain system. For example, cholinergic neurons in the ventral pallidum are surrounded by a dense neurotensin-containing fiber network, and ChAT neurons in the bed nucleus of the stria terminalis may receive alfa-MSH fibers. These neurons are supplied only occasionally by other peptidergic afferents. On the other hand, cholinergic neurons in the substantia innominata receive a heavy input from virtually all of the peptidergic systems studied. Electron microscopic studies confirmed the presence of NPY-containing terminals on ChAT cell bodies and light microscopic evidence suggests that these terminals originate at least partially from local NPY-neurons. Supported by USPHS Grant NS. 23945 and 17743.

365.7

ELECTROPHYSIOLOGY OF PUTATIVE CH6 CHOLINERGIC NEURONS IN THE LATERODORSAL TEGMENTAL NUCLEUS. S.J. Grant, D.A. Highfield* and T. Piser*. Dept. Psych., Univ. Del., Newark, DE 19716.

Ascending cholinergic pathways from the brain stem have long been associated with regulation of arousal, but the activity patterns of these neurons are unknown. This study examined the activity of putative cholinergic neurons (Ch6) in the Laterodorsal Tegmental nucleus (LDT).

Extracellular recordings from the pontine tegmentum, including the LDT, and Locus Coeruleus (LC), were made in anesthetized rats. Putative Ch6 neurons were identified in vivo by antidromic activation from the AV thalamus since 90% of this projection from the LDT is cholinergic. NADP-d staining (a positive marker for Ch5-6 neurons) indicated whether recording sites lay within clusters of cholinergic neurons. LC neurons were identified by standard neurophysiological and histological criteria.

55 neurons met standard criteria for antidromic activation from AV thalamus. 41% were within clusters of NADP-d positive neurons in the LDT, 49% were localized to the LC, while 9% were found in other areas. LDT neurons were 1) non-spontaneous (78%), or slowly firing (<1 Hz; 22%), and 2) had slow conduction velocities (0.8 $\pm$ 0.3m/s) which were nonetheless significantly faster than LC neurons (0.5 $\pm$ 0.4m/s; t=3.1, p=0.003). Studies are in progress examining the pharmacological responses of putative Ch6 neurons. Supported by NIH Biomedical Grant and UDRF (SJG), and UD Honors Program (DH).

365.8

CHOLINERGIC EXCITATION AND INHIBITION MEDIATED BY TWO DISTINCT K CONDUCTANCES IN MEDIAL PONTINE RETICULAR FORMATION. U. Gerber*, R.W. Greene, and R.W. McCarley, (Spon. J. Schildkraut), Harvard Medical School/ VAMC, Brockton, MA 02401

The best phenomenological model of REM sleep is evoked by microinjection of cholinergic compounds into the medial pontine reticular formation (mPRF) but the mechanisms responsible for these effects are unknown. Using an in vitro mPRF slice preparation from young rats we investigated the effects of the cholinergic agonist, carbachol on 27 neurons. Bath or puffer pipette applied carbachol evoked a slow depolarization associated with a conductance decrease in 75% of the cells, a hyperpolarization associated with a conductance increase in 15% and a biphasic (hyperpolarization- depolarization) response in 11% of the cells. These responses were observed in the presence of TTX. Under voltage clamp control, the reversal potential was determined to be more negative than -80mV for both the depolarizing and the hyperpolarizing responses. The I/V relationship for the depolarizing response was linear indicative of a voltage-insensitive membrane conductance. Further analysis of the hyperpolarizing response in 2 cases showed a voltage sensitivity between -100 and -50mV with inward rectification. Our results suggest that a decrease in a voltage insensitive K conductance and an increase in a voltage sensitive K conductance are responsible for the cholinergically-evoked depolarizing and hyperpolarizing response,respectively.

365.9

DEPOLARIZATION CONTROL CHOLINERGIC MUSCARINIC RECEPTORS: SELECTIVE EFFECT ON DIFFERENT NEURONAL CELL TYPES. R. Simantov and R. Levy.* Dept. of Genetics, Weizmann Inst. of Science, Rehovot 76100, Israel.

Muscarinic cholinergic neurons regulate many activities in the nervous system, including learning and memory. It is now established that cholinergic neurons degenerate in specific brain regions of patients with Alzheimer's type dementia. An abnormal behaviour may result however from changes in the activity rather than destruction of a given neuronal system. Thus, an increased expression of cholinergic phenotypes, like muscarinic receptors, may alter the function of various neuronal circuits. As neuronal activation regulates many developmental and functional processes, we have studied herein whether chemical depolarization modulates the expression of muscarinic receptors in cultured neurons. Continuous depolarization of a subclone of the NG108-15 hybrid cells (with KCl) increased the number of muscarinic receptors, monitored with ^{3}H-QNB, but short treatment had no effect. The calcium channel blocker verapamil enhanced the effect of KCl. Two rat brain cell lines, SC9 and WC5, that also bind ^{3}H-QNB, did not show an increased binding of ^{3}H-QNB upon chronic depolarization. The specificity of the receptors in the three cell types was therefore determined with the selective M-1 compound pirenzepine, and indeed SC9 and WC5 cells have higher affinity to pirenzepine than the NG108-15 cells. The physiological significance of this differential role of depolarization on the expression of different muscarinic receptors is discussed.

365.10

AF102B: A POTENTIAL DRUG FOR THE TREATMENT OF ALZHEIMER'S DISEASE (AD); FURTHER CHARACTERIZATION A. Fisher, R. Brandeis*, I. Karton*, Z. Pittel*, M. Sapir*, Y. Grunfeld*, G. Simon*, I. Rabinovitch*, S. Dachir*, A. Levy and E. Heldman*. Israel Institute for Biological Research, Ness-Ziona, ISRAEL

Cis-methyl-spiro(1,3-oxathiolane-5,3')quinuclidine (AF102B), a new centrally active M1 muscarinic agonist was suggested as a drug for the treatment of AD (Fisher et al, Soc. Neuroci., 13 Abs 184.12, 1987). Further characterization of this drug is described herein. AF102B has a ready passage through the blood-brain barrier as evidenced by its central selective effects and its pharmacokinetic profile. Whole body autoradiography in mice and tissue distribution in rats after iv injection of ^{3}H-AF102B showed that the cerebrum (rich in M1 receptors) absorbed higher radioactivity than the cerebellum (rich in M2 receptors). AF102B acts as a full agonist on the guinea-pig ileum preparation. However, unlike other full agonists, chronic administration (3 month, 5-100 mg/kg/day, po) of AF102B in rats, neither produce down-regulation of brain muscarinic receptors nor did it change its affinity towards these receptors. Central effects of AF102B were expressed in mnemonic processes in three types of animal models mimicking different aspects of AD. Thus in AF64A-treated rats, AF102B (0.2-1mg/kg, ip or po) reversed the cognitive impairments in passive avoidance (PA), or Morris water maze (MWM) and in 8-arm radial maze (1-5mg/kg) tasks. In addition, AF102B (3-5 mg/kg, ip) restored scopolamine-induced cognitive impairments in rats in the PA test, and improved the performance of old rats in the MWM task (1 mg/kg, ip) . All these new features of AF102B, together with its wide therapeutic index and its M1 selectivity as previously reported, indicate that AF102B may be an effective drug in the treatment of AD.
Supported by Snow Brand, Japan.

365.11

IMPACT OF PLASMA ANTICHOLINERGIC ACTIVITY ON COGNITIVE PERFORMANCE. O.J. Thienhaus, A. Allen*, J. Thoene*, F.P. Zemlan. Lab. of Geriatrics, Univ. of Cincinnati, College of Medicine, Cincinnati, OH 45267-0559.

An exploratory study yielded pilot data on the relationship between anticholinergic plasma activity and cognitive measures in geropsychiatric inpatients (mean age 59 years). Subjects received various psychotropic medications with known antimuscarinic properties. Plasma samples were obtained when patients were medication-free, and five to seven days after target dosage had been achieved. Samples were analyzed for total anticholinergic activity, using the tritiated quinuclidinyl benzilate ([3-H]-QNB) radio ligand binding assay (Tune, L., and Coyle, J.T., Psychopharmacology, 75:9, 1981). Results were expressed in units of atropine equivalence [pmole/ml].

Non-demented subjects (n=10) developed no cognitive dysfunction while their mean anticholinergic plasma activity reached 2314 pmole/ml (S.D.=1205). In demented patients, meeting diagnostic criteria for probable Alzheimer's disease (McKhann, G., et al., Neurology, 34: 485, 1984), aggregate cognitive rating scores revealed that cognitive performance ratings deteriorated an average of 18 percent while anticholinergic plasma activity increased to 2700 pmole/ml (S.D.=1957).

365.12

SPATIAL LEARNING IN THE WATER MAZE IS CORRELATED WITH CORTICAL ChAT IN NUCLEUS BASALIS LESIONED RATS. L.J. Thal[1,2], F.H. Gage[2], & R.J. Mandel[1,2]
1. Dept. Neurol. VAMC, SD, 92161, 2. Dept. Neurosci., UCSD, La Jolla, 92093

The degree of depletion of cortical choline acetyltransferase (ChAT) in rats with excitotoxic lesions of the nucleus basalis magnocellularis (NBM) has never been correlated with the extent of their learning and/or memory deficit. In Alzheimer's disease, the degree of dementia has been correlated with the loss of cortical ChAT. We report that acquisition and accuracy of performance in a water maze task is highly correlated with the reduction in cortical ChAT. F-344 rats (n=12) were anesthetized and bilaterally lesioned in the NBM (2 sites/side) with ibotenate in 2 stages 1 week apart. Three weeks post-surgery the 9 surviving lesioned and 8 sham control rats were tested in a water maze (1 trial block/day, 2 trials/block) for 5 consecutive days, followed by 2 days off, and then tested for 5 more days. The following week, rats were tested for 72 h retention (600s max.) of a passive-avoidance (PA) task (Jarvik 2 chamber box) after 2 initial training trials. Nine days after initial maze testing, the rats were tested for maze retention (1 trial block). The next day, after 1 trial block, the platform was removed and the rats swam for 90s for a spatial probe trial. The NBM lesioned rats had a significant reduction of cortical ChAT activity (mean 24% depletion). The NBM lesioned rats displayed a severe deficit in acquisition of the water maze task. Neither group displayed a retention deficit after a 9 day interval. The spatial probe trial revealed that the control rats remembered the spatial location far better than lesioned rats. Acquisition performance of the maze task was correlated with %ChAT depletion (r=.88). Spatial acuity, defined as the probability of being in the platform quadrant and the platform annulus during the probe trial, was correlated with %ChAT depletion (r=-.75). This is the first report of a significant correlation between a learning paradigm and degree of cortical ChAT depletion in the rat. These data suggest that the observed ChAT depletion is important for the behavioral deficit.

365.13

CHOLINERGIC NEURONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS IN THE ADULT RAT: A TOPOGRAPHICAL STUDY.
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Dept. of Pharmacology and Therapeutics, McGill University, Montreal (Quebec), Canada H3G 1Y6

We attempted to define the morphological landmarks of the rat nucleus basalis magnocellularis (NBM) based on the topographical organization and morphology of its cholinergic perikarya. The brains of 4 male Wistar rats were perfused and sequential 50 µm thick coronal sections of the entire NBM were cut, processed for choline acetyltransferase (ChAT) immunocytochemistry and studied with an image analysis system (Quantimet 920). Neuronal density as well as cell size and shape were assessed. Results indicated that in both the rostral and caudal thirds of the NBM the cholinergic neurons are distributed along a band following the ventro-medial edge of the globus pallidus. In the mid third of the nucleus cell distribution assumed a triangular outline and cell density was lower than in the other two thirds. However, cholinergic perikarya were larger in the mid NBM than in the rostral or caudal third where they were of equivalent size. Cell roundness measurements suggested that the shape of the ChAT immunoreactive nerve cell bodies remained constant throughout the entire rostro-caudal extent of the NBM. Thus the NBM can be readily identified within the rat basal forebrain based on the topographical distribution and density of its cholinergic neurons. (Supported by MRC and FRSQ).

BIOLOGICAL RHYTHMS: SYSTEMS I

366.1

ANNUAL VARIATION IN HYPOTHALAMIC LUTEINIZING HORMONE-RELEASING HORMONE, PLASMA AND PITUITARY LH, AND TESTOSTERONE PRODUCTION OF MIGRATORY GARDEN WARBLERS (Sylvia borin).
C. K. Bluhm, H. Schwabl*, E. Gwinner*, I. Schwabl*, and B.K. Follett*. Max-Planck-Institut für Verhaltensphysiologie Vogelwarte, 8138 Andechs, F.R.G., & Department of Zoology, University of Bristol, Bristol BS8 1UG England.

Garden Warblers show distinct annual cycles of testicular development and regression. Our goal was to determine how components of the hypothalamic-pituitary-gonadal system change temporally through phases of the annual cycle. We found distinct autonomous seasonal variation in hypothalamic GnRH-I content in warblers kept on constant daylength. GnRH-I content is low from December through March, but increases in April, and again from May to June. Of particular interest is the finding that GnRH-I content does vary in individuals during the 7 months preceding breeding, even in the absence of photoperiodic change.

Although pituitary LH content increased from March to April, levels varied only 2.5 fold during the study. Seasonal changes in plasma LH levels paralleled the changes in hypothalamic GnRH-I content; levels of both increased steadily from March to June. Increases in testicular mass occurred from March to April, and again from April to May. Increases in in vitro testosterone production paralleled increases in mass. Thus, in warblers a series of autonomous hormonal changes are important in regulating seasonal gonadal growth. Supported by the Alexander von Humboldt Foundation.

366.2

EFFECTS OF CHRONIC CLONIDINE ADMINISTRATION AND WITHDRAWAL ON CIRCADIAN ACTIVITY RHYTHMS IN RATS.
A.M. Rosenwasser, Dep't of Psychology, Univ. of Maine, Orono, ME 04469

The alpha-adrenergic agonist clonidine has been shown to alter mood, arousal, and activity. While acute administration is generally sedating, chronic administration can result in hyperarousal and irritability. Furthermore, a recent study showed that clonidine withdrawal results in a long-term behavioral depression. In light of the reported relationship between mood and activity disorders and circadian rhythm disturbances, I examined the effects of chronic clonidine administration and withdrawal on free-running circadian activity rhythms. After three weeks in constant dim light, female rats were treated with clonidine via the drinking water (5.0 ug/ml) for either two or three weeks, after which the drug was discontinued. During clonidine administration, free-running periods were shorter, circadian amplitudes were reduced, and overall levels of activity were lower, relative to baseline. When the drug treatment was withdrawn, animals treated for two weeks showed recovery of baseline rhythmicity, but animals treated for three weeks generally failed to recover, and continued to show altered rhythmicity for the duration of post-drug observations. These results suggest that central monoaminergic neurons may underlie the relationships between mood and activity states and circadian rhythmicity which have been previously reported.

366.3

CHRONIC CLORGYLINE DECREASES THE MAGNITUDE OF PHASE-SHIFTS IN WHEEL-RUNNING ONSET PRODUCED BY SUBSATURATING, MONOCHROMATIC LIGHT PULSES W.C. Duncan*, P.G. Sokolove, W. Orem and T.A. Wehr*(SPON: I. Wexler) Clinical Psychobiology Branch, NIMH, Bethesda, MD 20892

We have recently observed that chronic clorgyline (CLG), a monoamine oxidase inhibitor with antidepressant properties in humans, increases the period of wheel-running and alters the phase response curve to fifteen minute light pulses administered to Syrian hamsters. These experiments indicate that CLG differentially alters the response of the circadian pacemaker to light. For example, in the previous study, the magnitude of light-induced phase-delays was increased and the magnitude of light-induced phase-advances was decreased by chronic CLG treatment. The light intensity selected in the earlier study (28 μW.cm-2) was presumed sufficient to saturate the phase-shift response of drug-free hamsters; however in CLG-treated hamsters the intensity-response relationship between light and the phase-shift response is unknown. In order to examine the relationship between chronic CLG and responsivity of the circadian pacemaker to light, the phase-shift response to subsaturating, five minute pulses of monochromatic light was examined.

Group-housed hamsters were implanted with Alzet minipumps (Model 2002) containing either CLG (n=22 ; 2 mg.kg-1.day-1) or saline (n=18) for over four weeks. Individual hamsters were then transferred to cages and had free access to running-wheels, food and water. After about one week in LD 14.5:9.5, hamsters were exposed to continuous darkness(DD) for the next seven days. On day 8 hamsters were transferred to a light chamber where a five minute pulse of monochromatic light (505 nm, hb 10nm; .137 μW.cm-2) was delivered either at CT 13.5 or CT 18. Hamsters were then transferred back to individual cages and remained in DD for the remainder of the experiment. The change in the phase of wheel-running onset following the light pulse was calculated after the onset of wheel-running assumed a steady state. The magnitudes of phase-delays at CT13.5 and phase-advances at CT18 were significantly reduced by chronic CLG compared to saline. These data indicate that chronic CLG decreases the response of the circadian pacemaker to subsaturating visual stimulation of the circadian system.

366.4

DAILY INJECTIONS OF TRIAZOLAM INDUCE LONG-TERM CHANGES IN THE PERIOD OF THE CIRCADIAN ACTIVITY RHYTHM OF GOLDEN HAMSTERS. F.W. Turek and O. Van Reeth, Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL and Institute of Interdisciplinary Research, Free University of Brussels, Brussels, Belgium.

In the absence of a synchronizing light-dark cycle (i.e. during exposure to constant light or darkness), single injections of triazolam can induce permanent phase shifts in both behavioral and endocrine circadian rhythms in hamsters, and repeated injections at fixed circadian intervals can entrain the rhythm of locomotor activity. In addition to altering the phase of the circadian clock system, we have noted that following the termination of drug treatment, changes in the free-running period of the activity rhythm are often observed. In order to study this systematically, we examined the effects on period following the administration of triazolam or vehicle for up to 49 consecutive days. While daily injections of vehicle had no effect on period, daily injections of triazolam induced changes in the period of the activity rhythm that persisted for as long as 100 days following the termination of drug treatment. Thus, period as well as phase of a mammalian circadian clock can be altered by treatment with benzodiazepines.

366.5

EFFECTS OF ALPRAZOLAM ON THE CIRCADIAN SYSTEM OF GOLDEN HAMSTERS. P.C. Zee* and F.W. Turek (SPON: J. Joy). Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

The demonstration that the short-acting benzodiazepine, triazolam, can induce phase shifts of circadian rhythms, raises the possibility that alprazolam, a triazolobenzodiazepine with different therapeutic properties can also affect the circadian system of the golden hamster. Intraperitoneal injections of alprazolam 2 mg. or DMSO vehicle were administered at eight circadian times (CT) in both constant light (LL) and constant darkness (DD). Injections of vehicle alone had no consistent effect on the activity rhythm, whereas, alprazolam induced phase shifts of the activity rhythm that were a function of the CT of treatment in both LL and DD. However, the phase response curves (PRC) in LL and DD were notably different: 1. The amplitude of both phase advances and delays was of a smaller magnitude in DD compared to LL. 2. In LL, phase advances occured at CT6 and CT9, whereas in DD, advances were seen consistently only at CT9. Alprazolam induced phase delays at CT21 and CT24 in both LL and DD. The differences between the PRC's in LL and DD suggest that light plays a role in the phase shifting effects of alprazolam and these differences may prove useful in delineating the mechanism of action of the benzodiazepines on the circadian system of hamsters.

366.6

DIFFERENTIAL EFFECTS OF TRIAZOLAM ON THE CIRCADIAN CLOCK IN INBRED STRAINS OF GOLDEN HAMSTERS. M.M. Hotz and F.W. Turek. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

A single injection of the benzodiazepine, triazolam, is known to produce large phase shifts in both behavioral and endocrine circadian rhythms of an outbred stock of golden hamsters (Lak:LVG(SYR)). Some within-species variation in this response has been observed. In order to investigate the possibility of genetic sources of this variation, we have studied the response to triazolam in inbred strains of the golden hamster. Phase shifts in the locomotor activity rhythm of hamsters kept in constant light in response to injections of 2.5 mg of triazolam were determined in two strains: MHA/SsLak and LSH/SsLak. The general shape of the phase response curve to injections of triazolam in the LSH strain is similar to that of the outbred stock (both are normally pigmented), with advances at CT 3 (circadian time; CT 12 = activity onset) and CT 6, and phase delays at CT 18 and CT 21. However, the magnitude of the phase shifts are greater in the LSH strain. The albino MHA strain does not exhibit significant phase shifts when injected at any CT, although the increase in activity typically associated with triazolam injections is observed in this strain. The lack of an effect of triazolam on the circadian clock of albino MHA hamsters may prove important in elucidating the physiological mechanisms underlying the effects of benzodiazepines on circadian rhythmicity.

366.7

HYPOGLOSSAL NUCLEUS ACTIVITY DURING WAKING AND SLEEP STATES. C.A. Richard, R.R. Terreberry, R.C. Frysinger and R.M. Harper. Dept. of Anatomy and Brain Research Institute, UCLA, Los Angeles, CA 90024-1763.

Obstructive sleep apnea in humans may result from relapse of the tongue toward the posterior wall of the pharynx during REM sleep, since activity of the genioglossus muscle, the principal tongue protruder, greatly diminishes during that state. During waking and quiet sleep, genioglossal EMG activity phasically increases during each inspiratory effort. However, during REM sleep, both phasic and tonic activity decrease dramatically. The nucleus reticularis gigantocellularis (NGC) may hyperpolarize XII nerve motoneurons during REM sleep. Stimulation of the NGC causes IPSPs in somatic motor neurons only during REM periods. NGC neurons also demonstrate selectively greater activity during REM sleep. We hypothesize that hypoglossal motoneuronal activity will decrease during REM sleep and that NGC input underlies this decrease. Neuronal discharge was recorded from the hypoglossal nucleus in drug-free cats across all sleep and waking states. During REM epochs, hypoglossal activity showed periods of very low or no activity interspersed with periods of faster activity similar to rates in the awake state. Many of the faster REM firing rates coincided with phasic REM events. NGC stimulation elicited short-latency neuronal responses in the hypoglossal nucleus. The NGC may mediate state-specific effects on hypoglossal activity. (Supported by HL 22418-10)

366.8

SEX DIFFERENCES IN PARADOXICAL SLEEP IN THE RAT. J. Fang*, C. Lewis*, and W. Fishbein. Neurocognition Prog, CUNY, City College & Graduate Sch, N.Y. 10031.

Last year we reported on sex differences in the ultradian cyclicity of sleep in the mouse, and also reported that the sleep of males could be completely sex-reversed by prenatally stressing them. The findings were the first to indicate that sleep is sexually dimorphic. We have now extended our observations to the Sprague-Dawley rat.

Eight female and eight male animals were used. At 116.13 +/- 6.55 days of age, sleep-wakefulness cycles were recorded continuously over a 2-7 day period. The mean weight of the females was 65.28% of the males. Therefore, a second recording of the females at 268.63 +/- 11.26 days of age was taken. The females were then 86.45% of the male group weight.

The time spent asleep over 24 hrs. was the same in the two sexes. However, males spend significantly more time in Paradoxical Sleep (PS) than females ($F(1,14)=4.73$ $p<.05$) and this difference was most visible during the day (10am-8pm) ($F(1,14)=10.79$, $P<.005$), and even more pronounced in the 2nd run of the female group. The difference is totally accounted for by a greater number of PS episodes in the males ($F(1,14)=8.11$, $p<.01$), with the average duration of the PS episodes undiscernibly different. The greater amount of PS in males was not reflected in a significantly greater amount of Slow Wave Sleep in females.

The results are practically identical to our observations in the mouse, but the direction of the differences is just the opposite; namely, female mice have more PS than males whereas male rats have more PS than females. Thus, there are clear organizational differences in sleep between the sexes. We believe that a single hormonal agent may be responsible for the sexual differentiation of sleep, however, resolution of the differences between species will not be found in terms of a single hormone, but of androgen-receptor-mediated differences, most likely, in basal forebrain target neurons.

366.9

COMPUTER NETWORK RECONSTRUCTION OF PROJECTION POPULATIONS TO CHOLINOCEPTIVE REM SLEEP INDUCTION SITES.
J. Quattrochi, T. Hensch,* A. Mamelak* and J.A. Hobson. Laboratory of Neurophysiology, Harvard Medical School, Boston, MA 02115.

We define the precise multicellular network projecting to a discrete injection site within the anterodorsal pontine tegmentum which generates REM sleep when stimulated by carbachol fluorescent microspheres. This pharmacologically active retrograde probe and 3-D computer reconstruction of labelled cells permit the spatial dissection of afferent populations involved in the generation of REM sleep. Our observations in four adult cats indicate a labelled projection network within discrete nuclear boundaries of the gigantocellular, paralemniscal, lateral, central, pedunculopontine, and dorsolateral tegmentum, paramedian reticularis, dorsal raphe magnus, locus coeruleus, parabrachialis, and nucleus cuneiformis. Labelling intensity per unit volume and spatial topographical analysis indicate distinct patterned arrangements between anatomically defined cholinergic (Ach) and noradrenergic (NA) subgroup populations. We interpret these results within the context of whether (1) Cholinergic neurons project to the REM sleep induction site, and (2) REM sleep generation can be modeled structurally as the network intersection among Ach and NA projection populations. Supported by MH13923.

366.10

A TEST FOR CIRCADIAN VARIABILITY OF THE RESTLESS LEGS SYNDROME IN PATIENTS TREATED WITH OPIOIDS. W.A.Hening, A.S. Walters*, and S. Chokroverty*. Lyons VAMC, NJ 07939

Restless legs syndrome (RLS) is a sensorimotor disorder defined by paresthesias and motor restlessness. Its features include periodic movements in sleep, dyskinesias while awake (DWA), and sleep dysfunction (Walters & Hening, Clin Neuropharm 10:225). These symptoms, provoked by repose, are usually experienced at night. They may be powerfully suppressed by opioids, then reactivated by naloxone, suggesting the involvement of the endogenous opiate system in RLS (Hening et al., Neurology 36:1363). We developed a test for activation of RLS symptoms and used it to study circadian influences on symptom expression in 2 opioid-treated patients with RLS. At intervals during the day-night cycle, we required the patients to remain supine for 1/2 hour while we recorded bilateral ant. tibialis EMG. On half the trials, we gave 0.8 to 1.6 mg of naloxone I.V. This provocative test caused DWA in both patients that consisted of rapid leg flexions, most marked at hip and knee, that were sometimes quite periodic. Pt. 1, whose symptoms were poorly controlled, had DWA that were not aggravated by naloxone, while Pt. 2, who was asymptomatic on therapy, had DWA only after naloxone. In both patients, DWA appeared with shortest latency and highest frequency at night. This results suggests that RLS symptoms may be provoked by repose at all hours, but most easily at night while opioid therapy, even if it suppresses symptoms, may not change this tendency.

366.11

LOW SODIUM DIET ELEVATES PLASMA NOREPINEPHRINE (NE) AND IMPAIRS SLEEP PATTERNS IN MAN: A REPLICATION
M.V. Vitiello*, R.C. Veith*, D.D. Ralph* and P.N. Prinz. (Sponsor: K.F. Moe). Psychiatry and Behavioral Sciences and Medicine, University of Washington and Seattle and American Lake VAMC, Seattle, Washington 98195, USA.

Previously we reported that a low sodium diet significantly elevated plasma (NE) and disturbed sleep patterns in healthy young men, indicating that increased sympathetic nervous system (SNS) activity may affect sleep. In an attempt to replicate this finding, we studied the sleep and nighttime plasma NE levels of 9 healthy young men (23+.3 years) after four days of low (500mg/day) and normal (2,000mg/day) sodium diets presented in counterbalanced order. Sleep ratings and NE assays were made blind to condition. NE was significantly elevated ($p<.02$); sleep efficiency was lower ($p<.025$) with subjects awake longer ($p<.025$) and more often ($p<.01$); REM sleep was reduced ($p<.05$) and REM latency lengthened ($p<.05$) in the low compared to the normal sodium condition. Sleep latencies to Stages 1 and 2, time in bed, total sleep time and stage 3 & 4 sleep were not significantly different between conditions. NE correlated with total number of awakenings ($r=.75$, $p<.02$) and number of awakenings ≥ 1 minute ($r=.66$, $p<.05$) in the low sodium condition. These results confirm our original finding that increased SNS tone results in disturbed sleep. This may help explain the disturbed patterns of the healthy elderly, who exhibit elevated nighttime SNS activity.

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366.12

CEREBRAL GLUCOSE METABOLIC STUDY OF GENERALIZED ANXIETY DISORDER AS ASSESSED BY PET
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INTRODUCTION A study of regional cerebral metabolism of anxiety as assessed by positron emission tomography was performed. Twenty patients who met DSM-III-R criteria for generalized anxiety disorder (GAD) without panic attacks were compared with nineteen normal controls. Subjects were given 5 mCi of 18 FDG and told to fixate on a flashing light. Subjects were then scanned on a NEUROECAT II PET scanner. RESULTS There was a significant decrease of relative glucose metabolism on the left side of the cerebral cortex in GAD patients versus controls (significant hemisphere by group interaction, $F=5.41, d.f.=1,37, p=.03$). Basal ganglia structures such as the right putamen and left globus pallidus showed a significant decrease. Deep limbic structures such as amygdala, the hippocampus, and parahippocampus showed no significant change in GAD patients compared to controls. Thalamic structures showed no significant change in GAD.

366.13

SPECTRAL ANALYSIS OF PREMENSTRUAL TENSION SYMPTOMS.
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Premenstrual tension refers to cyclic variability of mood and physical symptoms in the late luteal phase of the menstrual cycle. Characteristics include irritability, depression, breast tenderness. Forty women diagnosed as premenstrual tension sufferers were studied during one assessment cycle and nine treatment cycles using dietary supplementation with essential fatty acids in a double blind crossover design. The subjects kept daily records of mood and somatic symptoms using a visual analogue scale throughout the study. Ovulation was verified by radioimmunoassay of estrogen and progesteron in blood samples drawn in the follicular and luteal phases of the cycle. The daily ratings were analyzed using a time series technique adapted for prospective mood ratings, where the underlying pattern is identified by spectral density analysis. The results showed that both adverse and positive moods displayed 28 day cyclicity. Patients varied in the number of symptoms with significant cyclicity. Treatment with essential fatty acids did not alter the degree of cyclicity of premenstrual tension symptoms.

366.14

OLFACTORY BULBECTOMY DISRUPTS CIRCADIAN RHYTHMS AND AGGRESSION IN MALE MICE.
A. R. Lumia*, B. Possidente*, L. Delemos*, L. Sterner*, M.Y. McGinnis and Martin H. Teicher. Depts. Psychology and Biology, Skidmore College, Saratoga Springs, NY 12866 Dept. Anatomy, Mt. Sinai Sch. Med., CUNY, New York, NY 10029, and Dept. of Psychiatry, Harvard Medical School and McLean Hospital, Belmont, MA 02178.

Depression in humans is often characterized by disruption of circadian rhythms, loss of sexual libido, and irritability. In rats, we have previously shown that olfactory bulb removal (OBX) produces similar symptoms, disrupting daily activity rhythms and copulatory behavior. These disruptions can be reversed by treatment with antidepressant, amitriptyline. In the present experiment using male SWR mice we found that OBX reduced the proportion of total wheel running activity during the peak phase (from .83+ .03 in sham to .74+ .04 in OBX) of a 12:12 cycle as previously described in rats. The most interesting finding, however, was a shift of 43 min in the period of a free running circadian rhythm for activity in OBX mice (23.89+/- .05 OBX vs 23.17+/- .10 sham; $p<.0001$). Aggression against castrated males was abolished by OBX. After surgery 9 of 12 shams and 0/12 OBX mice attacked castrated males. These data combined with our previous data from rats suggest that OBX rodents may serve as a useful model for agitated depression.

367.1

LONG-TERM SENSITIZATION IN APLYSIA INCREASES THE NUMBER OF PRESYNAPTIC CONTACTS ONTO THE IDENTIFIED GILL MOTOR NEURON L7. C.H. Bailey and M. Chen. Ctr. for Neurobiol. & Behav., Dept. of Anat. & Cell Biol., Neurol., & Psychiat., Columbia P&S, and NYSPI, NY, NY 10032.

To complement our morphological studies of identified sensory neuron presynaptic terminals, we have examined the effects of long-term sensitization on the structure of an identified postsynaptic target -- the gill motor neuron L7. Individual motor cells from control (N=2) and sensitized animals (N=4) were labeled with HRP and serially reconstructed. Preliminary l.m. observations suggest an increase in focal outpocketing of finger-like processes (spines) in sensitized cells. Quantitative ultrastructural analysis of 945 labeled profiles revealed an increase in the frequency of presynaptic contacts onto L7 processes in sensitized compared to control animals (0.45 ± 0.03 S.E.M. vs. 0.198 ± 0.02, $t=5.32$, $p< 0.01$). The number of synaptic contacts/μm^2 increases (1.72 ± 0.06 vs. 0.55 ± 0.09, $t=10.54$, $p < 0.001$) as does the incidence of multiple synaptic contacts onto the same postsynaptic profile (0.06 ± 0.008 vs. 0.016 ± 0.001, $t=3.8$, $p< 0.05$). Combined, these data indicate a striking upward shift following long-term training in the percentage of L7's surface area that is occupied by synaptic contacts (0.08 ± 0.002 vs. 0.02 ± 0.006, $t=11.8$, $p < 0.001$). These results are consistent with our observations of an increase following sensitization in sensory neuron synapses and provide additional support for the notion that changes in synapse number may represent a mechanism underlying long-term memory.

367.2

SEARCH FOR ADDITIONAL MOLECULAR SITES OF STIMULUS CONVERGENCE DURING ACTIVITY-DEPENDENT FACILITATION, A MECHANISM OF CLASSICAL CONDITIONING IN *APLYSIA* SENSORY NEURONS. T.W. Abrams Dept. of Biology & Inst. of Neurological Sciences, Univ. of Penn., Phila., PA 19104.

During conditioning of the withdrawal reflex in *Aplysia*, synaptic transmission from sensory neurons (SNs) of the CS pathway is strengthened through activity-dependent presynaptic facilitation. In activity-dependent facilitation, the SNs' activity and the accompanying Ca influx, triggered by the CS, enhance the SNs' response to facilitatory transmitter released by the US. Since synaptic facilitation in SNs is mediated at least substantially by cAMP, and since paired activity enhances the rise in cAMP produced by facilitatory transmitter, it has been suggested that the calmodulin-sensitive adenylate cyclase may be a molecular site of stimulus convergence between Ca influx, the signal from the CS, and transmitter, the signal from the US. Are there additional sites of activity-dependent enhancement downstream from the synthesis of cAMP? The spike broadening response in SNs, which contributes to presynaptic facilitation, is similarly enhanced by activity that is paired with facilitatory transmitter. In an initial investigation of downstream sites of activity-dependent enhancement, we measured the spike broadening response in experiments in which we bypassed the cyclase, substituting a rise in cAMP for transmitter. Brief elevations in cAMP were achieved by photolysis of "caged" cAMP (dimethoxynitrobenzyl cAMP). A UV light flash was given either immediately after a train of 5 spikes in a SN (Paired) or 4 sec after a spike train (Unpaired). Paired activity did not increase the spike broadening response to the cAMP transient. These preliminary results suggest that the dually-regulated cyclase may be the major site of stimulus convergence during this form of associative synaptic plasticity.

367.3

SEROTONIN PAIRED WITH SPIKE ACTIVITY IN SENSORY NEURONS OF APLYSIA PROLONGS FACILITATION OF SENSORIMOTOR SYNAPSES IN CULTURE. P.G. Montarolo* and S. Schacher (SPON: G. Clark). Ctr. Neurobiol. & Behav., HHMI, Columbia P&S, & NYSPI, New York, NY 10032.

Activity-dependent enhancement of heterosynaptic facilitation at sensorimotor synapses has been proposed to be the cellular mechanism underlying classical conditioning in Aplysia. To study further the molecular mechanisms underlying this synaptic plasticity, we have examined the interaction between spike activity in sensory neurons and facilitation produced by application of serotonin (5-HT) on the reconstituted sensorimotor synapse in culture.

We measured the amplitude of the excitatory synaptic potential (EPSP) evoked in motor cell L7 and 10 min later treated the cells with either a) control treatment; b) 5-HT puff; c) tetanus to sensory neuron (20 Hz for 2 sec); or d) tetanus plus 5-HT puff at 1 sec after onset of tetanus. The EPSP was measured at 1, 5, 10, and 15 min after the respective treatments. Each treatment significantly enhanced the EPSP after 1 min compared to controls (range of +27 to +42% compared to -14%, N=10). At 15 min, the EPSP following treatment with tetanus plus 5-HT ($+7 \pm 11\%$) was enhanced relative to treatment with 5-HT ($-39 \pm 3\%$), tetanus ($-26 \pm 3\%$) or controls ($-41 \pm 2\%$). One now needs to determine whether this prolongation of synaptic enhancement requires the temporal pairing of the two stimuli, and whether other facilitatory transmitters can substitute for 5-HT.

367.4

SEROTONIN SELECTIVELY PRODUCES LONG-TERM FACILITATION OF APLYSIA SENSORIMOTOR SYNAPSES IN CULTURE. S. Schacher and P.G. Montarolo*. Ctr. Neurobiol. & Behav. & HHMI, Columbia CPS & NYS Psychiatric Inst., New York, NY 10032.

Serotonin (5-HT), an important transmitter involved with behavioral sensitization of the gill and siphon withdrawal reflex in Aplysia, can produce both short- and long-term facilitation of the connections between the sensory and motor cells in culture. This synapse can also be enhanced for a short duration following tetanic stimulation of the sensory cells or brief application of small cardioactive peptide (SCP-B). We therefore examined whether SCP-B and tetanic stimulation, as 5-HT, can evoke long-term changes in sensorimotor synapses.

We first developed a protocol in which comparable short duration synaptic enhancement was evoked by 5-HT, SCP-B, and tetanic stimulation. After testing the amplitude of the excitatory synaptic potential (EPSP) evoked in motor cell L7, each culture was given one of the treatments 4 times at 20 minute intervals. Whereas 5-HT significantly enhanced the amplitude of the EPSPs when retested 24 hrs later ($58 \pm 11\%$, N=6), treatments with SCP-B ($7 \pm 10\%$, N=6) or tetani ($6 \pm 6\%$, N=6) did not significantly affect synaptic strength and were similar to the control untreated group ($4 \pm 6\%$, N=6).

These results, which parallel those from short- and long-term synaptic depression, suggest that the long-term modulation of this identified synapse may be evoked only by a subset of the neuromodulators that can produce short-term synaptic plasticity.

367.5

SEROTONIN (5-HT) CAUSES A PERSISTENT INCREASE IN PROTEIN PHOSPHORYLATION THAT IS TRANSCRIPTION-DEPENDENT: A MOLECULAR MECHANISM CONTRIBUTING TO LONG-TERM SENSITIZATION IN APLYSIA SENSORY NEURONS. J.D. Sweatt* and E.R. Kandel (SPON: N.L. Leith). HHMI, Columbia P&S, New York, NY 10032.

We have developed an intact-cell protein kinase assay for clusters of pleural sensory neurons based on the analysis of proteins labeled with ^{32}P and run on quantitative 2-D gels. A single application to these cells of either 5-HT (40 uM, 2 min) or 8(4 chlorophenylthio)-cyclic AMP (+ isobutylmethyl-xanthine, 100 uM ea., for 10 min), which produces short-term presynaptic facilitation, caused an increase in phosphorylation of 17 proteins (ranging in m.w. from 20 kD to 50 kD, and pI from 4.5 to 7.5). The increases range from 50% to 700%. Five pulses of 5-HT or application of 5-HT or cAMP for 2-hrs, which produces electrophysiologic effects lasting 24 hrs, caused an increase in phosphorylation of the same 17 proteins, but this increase now persisted for 24 hrs after stimulus washout. Addition of 10 uM anisomycin (a protein synthesis inhibitor) or 10 uM actinomycin D (an RNA synthesis inhibitor) during the 5-HT or cAMP application blocked the increase in protein phosphorylation observed at 24 hrs, without affecting the increase in phosphorylation in response to a 2 min 5-HT treatment. These data suggest that, in addition to their role in short-term facilitation, 5-HT and cAMP can recruit a long-term mechanism for a persistent increase in protein phosphorylation that is dependent for its induction on active translation and transcription.

367.6

SEROTONIN (5-HT) CAUSES CHANGES IN PROTEIN SYNTHESIS IN PLEURAL SENSORY NEURONS FROM APLYSIA. A. Barzilai, T.E. Kennedy, E.R. Kandel and J.D. Sweatt*, HHMI, Columbia Univ. CPS, NY, NY 10032.

The long-term facilitation (LTF) induced by 5-HT in Aplysia sensory neurons is blocked by the application of anisomycin (a protein synthesis inhibitor) or actinomycin D (an RNA synthesis inhibitor) during the 1.5-hr training period. This finding suggests that genes and proteins are required for long-term facilitation that are not needed for the short-term process. We therefore studied the effect of 5-HT on total protein synthesis in the pleural sensory cells by measuring incorporation of ^{35}S-methionine (^{35}S-met) into the TCA-precipitable fractions. Application of 5 um 5-HT during the training period (1.5 hrs) induced three temporally distinct changes in overall protein synthesis as resolved by 30-min ^{35}S-met pulses: 1) a trough at 30 min (a decrease of $32\% \pm 9\%$ SEM), 2) an early peak at 1 hr ($36\% \pm 16\%$), and 3) a second peak at 3 hrs ($94\% \pm 30\%$). Beyond the effects on overall incorporation, 5-HT also changes ^{35}S-met incorporation into specific proteins. Application of 5-HT causes a selective and transient increase at 30 min in the rate of synthesis of three proteins in two-dimensional gels. At 3 hours, these early changes disappear, but two new proteins show a selective increase in labeling. Actinomycin D blocks both the early and late specific changes in protein synthesis, suggesting that these changes are mediated at the transcriptional level. Results qualitatively similar to those obtained with 5-HT were also obtained with 100 um 8-(4-chlorophenylthio) cAMP plus 100 um IBMX.

367.7

AMINO ACID SEQUENCE OF A PROTEIN WHOSE NET RATE OF SYNTHESIS INCREASES IN ASSOCIATION WITH LONG-TERM SENSITIZATION IN APLYSIA. T.E. Kennedy, E.R. Kandel, M. Knapp, and J.D. Sweatt*. Ctr. for Neurobio. & Behav., HHMI, Columbia Univ., New York, NY 10032.

Long-term sensitization of the gill and siphon withdrawal reflex in Aplysia, produced by one or four days of training, is associated with a specific increase in the incorporation of ^{35}S-methionine into four proteins (Castellucci et al., Neuron, July, 1988). One of these, Aplysia protein #407, MW 52 kD, pI 4.7, is present in sensory neurons and can be identified on coomassie blue-stained preparative 2-D gels of Aplysia total CNS extract. This protein was isolated from preparative gels and sequenced using a gas phase microsequencer. We obtained 16 residues of amino acid sequence (XPTVYFKEEFGDDXAE). A sequence homology search in protein sequence databases revealed no homologous proteins. However, we similarly isolated and sequenced a comigrating protein from a crude cell lysate of cultured rat embryo fibroblasts obtaining 15 residues of amino acid sequence (XPAXYFXEQFLDXRA). The Aplysia and rat proteins are 64% identical at the amino acid level. We have synthesized oligonucleotides which correspond to all possible coding sequences of 7 residues in the Aplysia peptide (YFKEEFG). We are currently using this oligonucleotide mixture to screen lambda gt 10 cDNA libraries prepared from both Aplysia total CNS and Aplysia abdominal ganglion sensory and motor neurons.

367.8

FACILITATING STIMULI, 5-HT, ELECTRIC SHOCK, AND PHORBOL ESTER, CAUSE TRANSLOCATION OF PROTEIN KINASE C IN APLYSIA NEURONS. T.C. Sacktor and J.H. Schwartz, HHMI and Dept. of Neurology, Columbia Univ. Col. Phys. & Surg., New York, NY 10032

The dependence on Mg^{2+} of protein kinase C (PKC) binding to membrane is complex: it is blocked by physiological Mg^{2+} concentrations, but reversed by Ca^{2+} and phorbol ester. The translocation produced by phorbol ester is maximal at 3 mM Mg^{2+}. Changes in intracellular Mg^{2+} may be important in regulating PKC in cells; in any case, the concentration of Mg^{2+} must be optimized to obtain consistent data on the role of the kinase in facilitating Aplysia neurons. We examined unilateral sensitization of the tail-withdrawal reflex (mediated by pleural sensory neurons) with the protocol of Scholz & Byrne (Science 235: 685-687, 1987), shocking one side of the animal. Facilitation of sensory cells also is produced by serotonin or phorbol ester. We find that all of these facilitating stimuli activate PKC, translocating the enzyme from cytosol to membrane.

367.9

SENSORY NEURONS IN APLYSIA EXPRESS TWO ISOZYMES OF PROTEIN KINASE C (PKC) WITH HOMOLOGY TO VERTEBRATE β_1 and β_2. K. Kruger, T.C. Sacktor and J.H. Schwartz, HHMI, Columbia Univ. Col. Phys. & Surg., NY 10032.

Serotonin activates two protein kinases in Aplysia sensory neurons, the cAMP-dependent kinase by increasing cAMP and PKC by translocation from cytosol to plasma membrane. Both enzymes modulate transmitter release (see Sacktor et al., These Abstracts). We screened a pleural ganglion sensory cell library (provided by M. Knapp, P. Goelet, and E.R. Kandel) at low stringency using rat β_1 PKC cDNA (G. Hausey and B. Weinstein) as probe. DNA sequencing of the several clones obtained revealed the two different forms of the enzyme with different 3' ends, homologous to vertebrate β_1 and β_2. The constant regions of Aplysia PKC, however, have greater than 85% homology to vertebrate PKCs. Northern blots with Aplysia clones show both isozymes are encoded by single transcripts of similar (10 kb) size. Antibodies to PKC synthetic peptides are raised to determine cell specificity and subcellular localization of the two isozymes and to study regulation of expression in short- and long-term sensitization.

367.10

ORDER DEPENDENCE IN THE ACTIVATION OF ADENYLATE CYCLASE BY SEROTONIN AND Ca^{2+}/CALMODULIN: A POSSIBLE MOLECULAR MECHANISM FOR ASSOCIATIVE LEARNING IN *APLYSIA*. Y. Yovell* and T.W. Abrams (SPON: C. Stein). Dept. of Neurobiology, Weizmann Institute, Rehovot 76100, Israel and Dept. of Biology and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Adenylate cyclase has been suggested as a molecular site of convergence for Ca^{2+} and serotonin, the cellular representations of the CS and US in classical conditioning of the gill and siphon withdrawal reflex of *Aplysia*. By modifying a recently developed, perfused membrane cyclase assay, we were able to study the temporal interactions of brief pulses of Ca^{2+}/calmodulin and serotonin in stimulating adenylate cyclase from *Aplysia* CNS. The apparatus enabled continuous monitoring of cyclase activity and rapid application and termination of ligand stimuli. We found that the magnitude of adenylate cyclase activation by 6 sec pulses of Ca^{2+} and serotonin depended on their order of presentation: cyclase activation was 25% to 50% greater when the Ca^{2+} transient immediately preceded the serotonin transient (Forward pairing) than when they were given in the reverse order (Backward pairing). The magnitude of the Forward-Backward difference depended on the concentration of Ca^{2+} used and on the presence of calmodulin in the assay. When a non-hydrolyzable GTP analog was used, no Forward-Backward difference was observed. These results suggest that adenylate cyclase may provide a molecular mechanism underlying the two requirements for contiguity detection in classical conditioning: temporal proximity and order dependence.

367.11

SEQUENTIAL CHANGES OF IONIC CURRENTS DURING CLASSICAL CONDITIONING OF HERMISSENDA. C. Collin*, I. Lederhendler* and D. L. Alkon. (SPON.: M. Bak) Lab. of Molecular and Cellular Neurobiology, NINCDS-NIH, Rockville MD 20892

Reductions of voltage-dependent K^+ currents (I_A, I_{Ca}++-K^+) and a Ca^{++} current (I_{Ca}++) in Hermissenda Type B photoreceptors persist for days after classical conditioning (Alkon et al, Science, 215:693, 1982; Collin et al, Neurosci. Abstr., 1986). Here, we studied changes in these currents which depend upon increasing numbers of training trials. After 50 trials I_A, measured in ASW 24 hours later, was reduced by 17% in conditioned compared to control animals. I_{Ca}++-K^+ and I_{Ca}++ remained unchanged in these groups. Twenty-four hrs after 100 trials I_{Ca}++-K^+ (measured as a tail current in 300 mM K^+o, 5mM 4-AP, 100 mM TEA) was reduced by 40% in paired as compared to control animals. I_{Ca}++, however, was not significantly different for conditioned animals After 200 trials, I_{Ca}++ (measured in 300 mM K^+o, 5mM 4-AP, 100 mM TEA) was reduced 20% with a further reduction to 40% after 400 trials but with no aditional change of I_A and I_{Ca}++-K^+.

These results indicate that during classical conditioning, ionic membrane currents change sequentially as well as progressively.

367.12

PROTEIN AND mRNA CHANGES INDUCED BY ASSOCIATIVE CONDITIONING OF HERMISSENDA. T.J. Nelson and D.L. Alkon, Lab. of Molec.and Cellular Neurobiology, NINCDS/NIH, Bethesda, MD 20892 (Spon.: A.Sidhu).

Protein synthesis inhibition enhances Ca2+ -mediated reduction of the K+ currents which undergo persistent reduction with classical conditioning of Hermissenda (H) (Alkon et al., PNAS (1987)). To assess the role of specific proteins in associative memory, H were trained with paired light and rotation. 24 hr after training, eyes were dissected and incubated for 6 hr with mixed 3-H amino acids and 32P-phosphate, and proteins were analyzed by HPLC. Conditioning increased a 21 kDa phosphoprotein (previously found to be a C-kinase and CaM-kinase substrate) by 1.6-fold compared with random controls receiving unpaired light and rotation ($p<0.05$), and decreased a 169 kDa phosphoprotein to 0.3x normal levels ($p<0.001$). The sp.act. of 3-H incorporation into the 169 kDa protein was increased 5-fold ($p<0.05$), while the sp.act of the 21 kDa protein was decreased to 0.47 x normal levels ($p<0.001$). No differences were observed between random and naive controls. All the above changes were significantly correlated with the degree of learning ($p<0.01$ to $p<0.05$).

In a related experiment, eyes from trained H were incubated with 32P-phosphate and mRNA was isolated and analyzed on agarose gels. The mRNA showed changes which paralleled the protein changes for the 21 and 169 kDa proteins. Thus, associative learning in H changes the synthesis rates of specific phosphoproteins within H eyes. These results are consistent with previous results demonstrating a correlation of mRNA levels with learned behavior in intact H up to 4 days after conditioning.

367.13

MODEL OF HERMISSENDA TYPE B PHOTORECEPTOR RESPONSE BASED ON PRINCIPAL SOMA CURRENTS. M. Sakakibara, S. Usui, H. Ikeno and D.L. Alkon, Dept. Inf. & Comp.Sci., Toyohashi Univ. of Tech., Toyohashi 440 JAPAN and Lab. of Molec.& Cell. Neurobiol., NINCDS, NIH, Bethesda, MD 20892.

To further understand the functional significance of persistent ionic current changes induced by Hermissenda classical conditioning, a model of the type B cell was formulated from Hodgkin-Huxley equations for three principal soma currents: I_A, ICa^{2+}-K^+, and I Na. Previous measurements demonstrated that on days after classical conditioning, but not control procedures, I_A and I Ca^{2+}-K^+ remain significantly reduced (Alkon et al.,1982, 1985),while the long-lasting depolarizing response (LLD) to light was enhanced and prolonged (West et al., Farley & Alkon, 1982). Based on the present model the observed enhancement of the LLD was shown to arise quantitatively from the observed reductions of I_A and I $Ca^{2+}K^+$ following conditioning. The model was also effective in producing quantitative estimations of actual ionic currents. Biophysical isolation of I_A and I Ca^{2+}-K^+ was predicted from differences in the activation and inactivation characteristics of these two currents. These predictions were confirmed by model responses to cycles of triangular waveform commands (±60 mv, holding potential at -60 mv). Triangular commands at 0.1 sec/cycle and 10 sec/cycle activated only I_A and I Ca^{2+}-K^+ respectively, the isolated currents closely resembling currents isolated by selective pharmacologic blockade (with 4-AP and TEA). Addition of other more minor soma ionic currents (e.g.,I Ca^{2+}) will be necessary for more exact simulation of the type B photoresponse particularly as it is progressively modified during acquisition of classically conditioned behavior.

PAIN PATHWAYS

368.1

MECHANISMS IN CENTRAL POST-STROKE PAIN (CPSP) - A CLINICAL STUDY J. Boivie, G. Leijon and I. Johansson. Depts of Neurology and Radiology, Univ. Hosp., Linköping, Sweden.

Central pain can be induced by thalamic as well as extrathalamic cerebrovascular lesions (CVL). To elucidate the mechanisms of CPSP the present study investigated to what extent the CVL engages the thalamus in patients with CPSP, which other locations of CVL can cause CPSP, if the pain differs according to the location of the CVL and which neurological symptoms and signs the patients (pts) have in addition to pain.

Patients and Methods. Examinations including quantitative sensory tests were done of 20 men and 7 women.

Results. The lesions involved the thalamus in 33 % of the pts. In 27 % and 22 %, resp., the CVL were located infratentorially and suprathalamically. The pain was usually burning, aching, pricking or lacerating with some differences due to CVL location. It was increased by various stimuli. Abnormal temperature sensibility was the only sign common to all pts. 93 % had some hypertensitivity to cutaneous stimuli. Hypaesthesia to touch and vibration was found in 52 % and 41 %, resp. 48 % had pareses.

Conclusion. The results indicate that the crucial factor for CPSP is a lesion affecting temperature (and possibly pain) sensibility. It appears likely that the spinothalamic tract or its relay or thalamocortical projections is involved. (SPON: ENA)

368.2

TOPOGRAPHY OF LATE SOMATOSENSORY EVOKED POTENTIAL COMPONENTS IN HUMANS: CUTANEOUS HEAT STIMULI VERSUS ELECTRICAL NERVE STIMULI. **R.-D. Treede*, S. Kief*, T. Hölzer*, B. Bromm*** (SPON: S.N.Raja). **Inst. Physiology, Univ. Hosp. Eppendorf,D-2000 Hamburg 20, F.R.G.**

Short cutaneous heat stimuli elicit late and intralate somatosensory evoked cerebral potentials (SEPc) correlating with first and second pain due to Aδ- and C-fiber activation (B. Bromm and R.-D. Treede, Exp. Brain Res., 67: 153, 1987). The late SEPc were compared with responses to electrical nerve stimuli (SEPn) which activate predominantly Aα- and Aβ- fibers.

In 24 subjects EEG was recorded from 14 electrodes over both hemispheres (bandpass 0.1-70 Hz). SEP were averaged over 80 stimuli each. Both hands and the left foot were tested in balanced order with CO_2-laser pulses (20 ms, 0.75-1.5 times pain threshold, hairy skin sites) and constant current stimuli (0.2 ms, 0.75-1.5 times motor threshold, median and tibial nerves).

In the upper limb both stimulus types evoked a common component sequence: a negativity N1 with a maximum over the contralateral somatosensory cortex was followed by a negativity N2 in central leads and a large positivity P2 in centro-parietal midline leads. SEPc peak latencies (170, 250, 390 ms) were about 100 ms longer than those of SEPn (70, 140, 260 ms) due to heat conduction to cutaneous receptors and slower nerve conduction velocity. N1 of SEPc was more lateral and N2 and P2 of SEPc more posterior than corresponding SEPn components. After stimulation of the lower limb all latencies were prolonged by 20-30 ms.

In spite of the different fiber spectrum activated by cutaneous heat and electrical nerve stimuli and the different projection in the spinal cord, the topography and inter-peak latencies indicated similarities in cerebral processing.

368.3

INVERSE RELATION BETWEEN HEAT PAIN THRESHOLD AND RATE OF TEMPERATURE RISE: THE REACTION TIME ARTEFACT. David Yarnitsky* and Jose Ochoa (SPON. Rose Dotson). Good Samaritan Neurological Sciences Center, Portland, Oregon, 97210 and Department of Neurology, Rambam Medical Center, Haifa, Israel.

Afferent impulse frequency varies with the rate of rise of a stimulus: the faster the increase in stimulus energy, the higher the frequency of firing for a given amount of energy (Darian-Smith et al 1979). This predicts that, for a given energy level, subjective magnitude will be higher the faster the stimulus reaches that level, and explains why the steeper the stimulus ramp the lower is the threshold for perception (Grindley, 1936; Hensel, 1952; Kenshalo et al, 1968).

However, the opposite has been reported for heat pain (HP) in man (Croze and Duclaux, 1978; Pertovaara and Kojo, 1985). We examined research reports so claiming and found that artefactual participation of reaction times (RT) might explain the paradox.

Our study compared HP thresholds through a method that involves RT participation, vs. those obtained by-passing RT. We found, in 16 volunteers, that if RTs are by-passed, thresholds decrease as the rate of temperature rise increases ($p<0.001$). A significant fall in HP threshold was demonstrated at all 3 rates used when omitting the RT artefact from threshold reading ($p=.018$, <0.001, <0.001).

368.4

SENSATIONS EVOKED BY SELECTIVE INTRANEURAL MICRO - STIMULATION OF C NOCICEPTOR FIBRES IN HUMAN SKIN NERVES. J. Ochoa and E. Torebjörk* Good Samaritan Neurological Sciences Center, Portland, Oregon, 97210 and Department of Clinical Neurophysiology, Academic Hospital, Uppsala, Sweden.

Microneurography of 36 identified C polymodal noci - ceptors supplying glabrous and hairy skin in awake human volunteers was supplemented with intraneural microstimulation (INMS). Cognitive attributes of ele - mentary sensations evoked by INMS at liminal intensity for perception were estimated psychophysically for subjective quality and localized projection.

There was excellent matching of physiological unit type (C polymodal nociceptor) with subjective quality of evoked sensation (dull pain). Further, there was remarkable spatial matching of unitary receptive field of C nocicep - tors with projected field of the elementary sensation evoked at liminal stimulus intensity.

While matching of subjective quality and localization with physiological type and receptor location gives evidence that unitary C nociceptor channels were selec - tively activated during INMS, the results also directly attest that C polymodal nociceptors from human skin evoke delayed dull pain, fairly accurately projected to a defined locus of skin.

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368.5

TWO COMPONENTS OF PAIN SENSATION IN RESPONSE TO COLD STIMULATION OF HUMAN TEETH. K.-D. Kniffki, E. Jyväsjärvi*, M.K.C. Mengel* and A. Stiefenhofer*. Physiologisches Institut der Universität, D-8700 Würzburg, FRG.

In animals it has recently been demonstrated, that cold stimulation of teeth elicits quite distinct responses in intradental Aδ- and C-fibres (Jyväsjärvi, E. and Kniffki, K.-D., J. Physiol. 391: 193 - 207, 1987). The aim of the present study was to evaluate, whether these different response properties of single intradental Aδ- and C-fibres are reflected in distinguishable pain sensations in humans.

Randomly adjusted cold stimuli between +25 °C and -40 °C lasting 2.5 min were applied to 13 upper central incisors of ten volunteers. The subjects had to rate the magnitude of their pain sensations using a verbally anchored 50-point scale (category partitioning), and afterwards described their sensations qualitatively using a list of descriptors.

Almost all subjects rated two components of pain. The magnitude of both pain components was dependent on the temperature of the stimulating individually adapted thermode. The first component had a mean threshold of 11 ± 3.6 °C, a latency of less than one second, lasted only a few seconds and was described as sharp, shooting and well localized. The average peak ratings are fitted rather well by a Steven's power function: $y = 2.58 \cdot |T - T_o|^{0.71}$, (r = 0.98). The second component had a mean threshold of 5 ± 2.8 °C, a latency of 10-20 s, often lasted some minutes and was described as dull, burning and poorly localized. The average ratings are fitted by $y = 2.02 \cdot |T - T_o|^{0.62}$, (r = 0.93).

Assuming that the response behaviour of human intradental Aδ- and C-fibres is similar to that of the cat, it is concluded that the first pain component evoked by cold stimulation of teeth is elicited by activation of Aδ-, and the second pain component by C-fibres.

368.6

BRANCHED Aδ- AND C-FIBRES TO BOTH PERIODONTAL LIGAMENT AND DENTAL PULP. M.K.C. Mengel*, E. Jyväsjärvi* and K.-D. Kniffki (SPON: C. Vahle-Hinz). Physiologisches Institut der Universität, D-8700 Würzburg, FRG.

Electrophysiological experiments were performed to study the responses of afferent Aδ- and C-fibres supplying both periodontal and pulpal tissues of the cat canine teeth. Recordings were made from single fibres, isolated from the inferior alveolar nerve in anaesthetized cats. They were identified by electrical current pulses applied to the periodontal space, the dental pulp, and in some experiments also to the nerve trunk. In addition, some of the fibres were tested with heat, cold, potassium-chloride and mechanical stimuli applied to tooth and periodontium.

Fifteen of 260 identified slowly conducting periodontal fibres were also activated by electrical stimulation of the dental pulp. Eleven of them were classified as C- and four as Aδ-fibres. The response characteristics of the fibres to non-electrical stimulation of the periodontium were similar to those of slowly conducting fibres innervating the periodontium exclusively (Jyväsjärvi, E., Kniffki, K.-D. & Mengel, M.K.C., Progr. Brain Res. 74: xx - yy, 1988). But, in addition, responses were also evoked by stimulating the pulp with heat, cold, potassium-chloride and drilling. The receptive fields of single C-fibres, determined with a dental probe, were located deep in pulpal and periodontal tissues. In a few cases, after determining the branched fibre's responses, the pulp was completely removed and the responses to stimulation of the periodontal ligament were found to persist.

It is concluded, that there exist some slowly conducting fibres in the inferior alveolar nerve, that branch to innervate more than one oral tissue as it has been shown in other peripheral nerves.

368.7

ANALYSIS OF THE ACTIVITY OF PRIMARY AFFERENT NEURONS IN A MODEL OF NEUROPATHIC PAIN IN THE RAT. K.C. Kajander and G.J. Bennett. NAB, NIDR, NIH, Bethesda MD 20892.

We have developed a model of neuropathic pain in the rat for which we have reported behavioral evidence of hyperalgesia, allodynia and spontaneous pain (Bennett and Xie, Pain 33:87-108, 1988). In this model, nerve injury is produced by ligatures tied loosely around the sciatic nerve. Behavioral evidence of neuropathic pain appears by the second day after nerve injury. We have now looked for changes in the activity of primary afferent neurons one day after the injury.

Rats were anesthetized with pentobarbital and paralyzed with gallamine triethiodide (Flaxedil-FLAX). The sciatic nerve was exposed and two bipolar stimulating electrodes were placed 10 mm apart, one proximal and one distal to the injured area. Recordings were made from the dorsal rootlets of spinal segments L4-6.

More than 200 individual sciatic axons were classified according to conduction velocity as Aß, Aδ or C fibers. Approximately 90% of Aß fibers and 65% of Aδ fibers were activated only from the proximal electrode. In contrast, 85% of C fibers were activated from both the proximal and distal electrodes. The receptive field charactertistics of 19 of the Aδ and C fibers were identified (nociceptive = 14, low threshold mechanoreceptive = 5). Additionally, 44 of 185 Aß and Aδ fibers exhibited non-evoked activity. Supplemental doses of FLAX (3mg/kg) increased the discharge frequency of many fibers with ongoing activity and also activated some previously silent fibers. The incidence and duration of the FLAX effect was highly variable. These data indicate that within one day this injury preferentially affects conduction in myelinated fibers across the ligated area.

368.8

THE DEACTIVATION OF C-FIBER NOCICEPTIVE RECEPTORS FOLLOWING INTRADERMAL INJECTION OF THE CAPSAICIN ANALOGUE NE-21610. R.H. Cohen, J.N. Campbell, A.A. Khan* and R.A. Meyer, Dept. Neurosurgery, Johns Hopkins Univ., Baltimore Md 21205

The potency, specificity, and time course for excitation and deactivation of cutaneous nociceptors following i.d. injection of the capsaicin analogue NE-21610 was determined in neurophysiological experiments in anesthetized monkey. Single fiber recording techniques were used to monitor the heat and mechanical responsiveness of primary afferents before and after 30 µl injections into the receptive field (RF). Four C-fiber nociceptors received a 0.3 µg dose which resulted in: (1) a brief (10 s) discharge, (2) a period (5-120 min) of complete insensitivity, and (3) a subsequent recovery. The evoked discharge after injection of 3 µg into the RF was similarly brief, however the deactivation lasted more than 240 min. In comparison, a 3 µg injection of capsaicin (n=5) produced a deactivation that lasts from 5 to 30 min. Injections adjacent to the RF of C-fiber nociceptors resulted in a small response (10-20 discharges), but no deactivation. In addition, other C-fiber afferents whose RF could not be located with mechanical stimuli were activated by injections. These afferents could be chemospecific. A 3 µg dose of NE-21610 did not activate or alter the responsiveness of A-fiber nociceptors (n=4) or low-threshold mechanoreceptors (n=2). These results suggest that, in comparison to capsaicin, the selective deactivation of C-fibers by NE-21610 is at least 10 times more potent and long lasting.

368.9

C-FIBER NOCICEPTORS IN MONKEY ARE SENSITIZED BY BRADYKININ ANTAGONISTS. A.A. Khan*, S.N. Raja, R.A. Meyer, J.N. Campbell, & D.C. Manning*, School of Medicine and Applied Physics Lab., Johns Hopkins U., Baltimore, MD 21205. U.S.A.

We previously demonstrated that the endogenous peptide, bradykinin (BK), sensitizes C-fiber nociceptors. To determine whether the B_1 or B_2 bradykinin receptor mediates this sensitization, we examined the effects of specific B_1 and B_2 receptor antagonists on the response properties of C-fiber nociceptors. Standard single-fiber techniques were used to record from 19 cutaneous C-fiber nociceptors responsive to mechanical and heat stimuli (CMHs). A fixed dose (10^{-8} moles in 10 µl neutral saline) of BK, the B_1 antagonist [desArg^9,Leu^8]BK, or the B_2 antagonist DArg[Hyp^3,DPhe^7]BK was injected intradermally into the receptive field of the nociceptor. The response to heat of the CMHs before and after drug administration was tested with a sequence of stimuli ranging from 41 to 49°C. Both antagonists, as well as BK, sensitized the CMHs to heat stimuli. The normalized response (i.e., the total response to the heat sequence after injection divided by the response before) was 1.8 ± 0.1 (n=10), 1.5 ± 0.1 (n=4), and 1.5 ± 0.2 (n=5) for BK, and the B_1 and B_2 antagonists, respectively. The vehicle injection had no effect on the thermal response. These results indicate that BK analogs, with antagonist properties in smooth muscle preparations, sensitize nociceptors. This suggests that BK and the BK analogs tested do not sensitize nociceptors through classical B_1 or B_2 bradykinin receptors.

368.10

ALTERED PREPROTACHYKININ GENE EXPRESSION IN TRIGEMINAL AND CERVICAL DORSAL ROOT GANGLIA IN RESPONSE TO SUBARACHNOID HEMORRHAGE.

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Massachusetts General Hospital, Boston, MA, 02114.

Substance P (SP)-containing projections from trigeminal and dorsal root (DRG) ganglia innervate cerebral vessels and may be involved in the transmission of pain information associated with blood in the subarachnoid space. We monitored neuropeptide levels in the cerebral arteries, trigeminal ganglia and DRG, as well as preprotachykinin mRNA levels in trigeminal ganglia, following the intracisternal injection of blood. Marked, 50% decreases in basilar artery SP levels were found within 4 h. SP in the DRG was depleted at 48 h and persisted for 7 days. The middle cerebral artery and circle of Willis were exposed to less blood and normal SP levels were maintained. In trigeminal ganglia, levels of SP were increased at 24 and 48 hrs. Preprotachykinin mRNA levels, as assessed by northern analyses, were also elevated in the trigeminal ganglia at 48 h. To investigate the pathophysiological mechanism in vitro, cultured F-11 cells (neuroblastoma x dorsal root ganglion) were exposed to hemoglobin (Hb). At 24 hrs, Hb caused a dose dependent reduction in SP in these cells. These results are consistent with blood-induced alteration of preprotachykinin gene expression in cerebrovascular sensory fibers, and implicate these fibers in the pathophysiology of subarachnoid hemorrhage. Supported by NS08166, NS10828, The McKnight, Sloan and American Parkinsons Disease Assns.

368.11

RECEPTIVE FIELD PLASTICITY OF DORSAL HORN CELLS: A COMPARISON BETWEEN MULTIRECEPTIVE AND NOCIRECEPTIVE NEURONES. J.M.A. Laird* and F. Cervero* (SPON: European Neuroscience Association) Department of Physiology, University of Bristol, Medical School, Bristol, U.K.

It is known that the receptive field (RF) size of some dorsal horn neurones can increase following a brief period of noxious stimulation. We have now investigated such RF changes of Multireceptive (Class 2) and Nocireceptive (Class 3) neurones in the sacral dorsal horn of the rat. The conditioning stimuli were 8N pinches delivered to the neurone's RF on the tail over a period of up to three hours, and/or electrical stimulation of A and C fibres from the tail. RF areas were measured and mechanical thresholds determined before, during and after these stimuli.

Following a single noxious pinch the RFs of all Class 2 neurones increased in size to include skin regions well away from the stimulated point. In addition, these cells were more excitable and their mechanical thresholds were decreased. Similar changes were seen after electrical stimulation, as has been previously described.

In contrast, Class 3 neurones exhibited much smaller increases in RF size which were restricted to the immediate area around the noxious pinch. Mechanical thresholds fell and excitability increased in some neurones but to a lesser degree than in the Class 2 cells. In about 50% of the cells several pinches were needed before changes were evident.

We conclude that RF changes in Class 3 cells following noxious stimulation can be explained by sensitisation of primary afferents whereas those observed in Class 2 neurones include an important central component. This shows fundamental differences in the processing of nociceptive information by Multireceptive and Nocireceptive cells.

368.12

SEGREGATION OF COARSE AND FINE GLOSSOPHARYNGEAL AND VAGAL AXONS IN THE NUCLEUS TRACTUS SOLITARII. F. Torrealba*, A. Claps* and F. Calderon* (SPON: N. Inestrosa). Dept. Physiol. Sci., Catholic Univ., Santiago, Chile.

In normal adult cats we studied with the Fink-Heimer method the distribution of degenerating glossopharyngeal afferents in the nucleus tractus solitarii (NTS), after the unilateral removal of the petrosal ganglion. At short survivals only a fine fiber component was present mainly in dorso-medial and commissural regions. At longer survivals fine fibers tended to disappear and a coarse component predominated, mainly in the lateral subnuclei. To support these findings we placed WGA-HRP injections in medial or lateral or commissural NTS regions and studied the distribution of sizes of the retrogradely labeled neurons in the petrosal and nodose ganglia. Medial injections produced bilateral label of small cells in both ganglia and large cells in the nodose ganglia. Lateral injections labeled small and large cells only in the ipsilateral petrosal ganglion. Commissural injections bilaterally labeled small cells in both ganglia.

These findings indicate that fine and coarse fibered inputs from visceral receptors differ in their central distribution and that they may participate in different central pathways.

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POSTSYNAPTIC MECHANISMS III

369.1

INTERACTIONS AND POSSIBLE SECOND MESSENGER SYSTEMS INVOLVED IN NEUROTRANSMITTER RESPONSES IN THE THALAMUS. David A. McCormick. Yale University School of Medicine.

ACh and NE are known to both decrease a resting gK, while ACh and the $GABA_B$ agonist, baclofen, can both activate a gK, in thalamic neurons. The possibility that these actions are achieved through the same ionic channels and/or second messenger systems was investigated using intracellular recordings in relay neurons of the guinea pig lateral geniculate nucleus, in vitro. Sequential application of ACh, NE and baclofen revealed that the ACh and NE-induced inward currents, as well as the ACh and baclofen-induced outward currents, displayed non-additivity indicating that these three transmitters converge upon only two gKs. Application of the muscarinic antagonist scopolamine blocked the ACh responses, but not those of NE or baclofen. LGNd neurones exposed to pertussis toxin (in vivo) displayed (in vitro) only the inward currents in response to ACh and NE and no response to baclofen. Similarly, intracellular injection of GTP-gamma-S resulted in a large hyperpolarization and blocked the outward currents induced by ACh and baclofen, but not the inward currents induced by ACh and NE. Intracellular injection of the calcium chelating agent BAPTA blocked the medium AHP, but not the ACh and NE responses. These results indicate that ACh and GABA activate the same gK in thalamic neurones through a pertussis toxin sensitive G-protein, while NE and ACh decrease the same gK through an as of yet unknown second messenger system.

369.2

ACTIVATION OF PROTEIN KINASE C REDUCES $GABA_A$ MEDIATED CHLORIDE CONDUCTANCE. A.Stelzer* and R.K.S.Wong. Department of Neurology, Columbia University, New York, N.Y. 10032.

There is increasing evidence that ligand-gated receptor-channel complexes are regulated by protein phosphorylation. The observation that $GABA_A$ receptor function is maintained by phosphorylation factors (Neurosci. Abstr. 13, 279.7) prompted studies to identify putative protein kinases involved in the regulation of $GABA_A$ receptor function. We examined the action of phorbol 12-13 dibutyrate on GABA-mediated chloride currents in acutely isolated cells prepared from the CA1 region of the guinea-pig hippocampus (Kay and Wong, 1986). Whole-cell voltage-clamp experiments were performed at room temperature, cells were clamped at -10mV. GABA (200 μM) was applied by short pressure pulses (20-80 ms). Application of phorbol 12-13 dibutyrate (200 nM) via perfusate reduced the peak-current amplitude of the GABA-mediated outward currents to 69.5% $\pm$ 4.1 (N=10) measured 5 min following phorbol 12-13 dibutyrate application. 400 nM of phorbol 12-13 dibutyrate reduced GABA currents to 45.1 $\pm$ 7.2 (N=5) whereas 4α Phorbol had no effect on GABA responses. Following washout of phorbol 12-13 dibutyrate GABA currents recovered partially (81.5% $\pm$ 5.2, N=5, compared to 63.8 % $\pm$ 4.7 during phorbol 12-13 dibutyrate application in these 5 cells). These data indicate that activation of protein kinase C by phorbol 12-13 dibutyrate reduces $GABA_A$-mediated chloride conductance. Taken together with previous findings (Neurosci. Abstr. 13, 279.7) it may be speculated that the $GABA_A$ receptor is regulated by several protein kinases, as reported for the nicotinic Ach receptor (comp. Huganir and Greengard, TIPS, 1987).

Supported by grants from NIH and Klingenstein Foundation.

369.3

MODULATION OF ELECTROTONIC COUPLING BETWEEN HIPPOCAMPAL NEURONS BY NMDA AND C-KINASE. **M. O'Beirne and B.A. MacVicar. Neuroscience Research Group. University of Calgary, Calgary, Alberta. T2N 4N1**

Electrotonic coupling and dye coupling has been observed between hippocampal neurons in brain slices and in dissociated tissue culture. We have previously shown dye coupling to be correlated with electrotonic coupling in tissue culture. We have examined the modulation of electrotonic coupling between hippocampal neurons by determining the extent of dye-coupling in culture and in slices in the presence of NMDA and C-kinase activators.

In control, 23% (n=35) of cultured hippocampal neurons were dye coupled. Application of NMDA(10^{-4} 10^{-3}M) abolished dye-coupling (n=28) as did TPA (50nM, n=21) a phorbol ester which activates C-kinase. In slices, dye coupling was also abolished by TPA (n=15 vs 50%, n=16 in control). Preincubation of slices in H-7 (100 µM), a C-kinase inhibitor prevented TPA uncoupling. NMDA application appeared to be toxic in brain slices so we indirectly activated NMDA receptors by incubating slices in Mg++ free solution and dye coupling was abolished (n=16). These findings indicate that electrotonic coupling between hippocampal neurons is dynamiccally modulated.

369.4

FORSKOLIN ACCELERATES DESENSITIZATION OF 5-HT_3 RECEPTORS IN MOUSE HIPPOCAMPUS AND NG108-15 CELLS. J.L. Yakel* & M.B. Jackson, Dept. of Biology, UCLA, Los Angeles, CA.

5-HT induces rapid currents in hippocampal neurons (Yakel et al, J. Neurosci. 8:1273) and NG108-15 cells (Christian et al, Brain Res. 147:261). Studies in NG108-15 cells show that this current is carried by sodium and potassium ions. ICS 205-930 (1 nM), a selective 5-HT_3 antagonist, blocked these responses. 2-methyl-5-HT, a selective 5-HT_3 agonist, partially activated these responses. The magnitude of the response produced by 2-methyl-5-HT (50 uM) was 8% of the 5-HT response (also 50 uM). This suggests that the rapid 5-HT response in both cell types is mediated through the 5-HT_3 receptor.

The response in both cell types completely desensitizes, with a biphasic time course. Quantitative analysis of the time course of desensitization in NG108-15 cells yielded average time constants of .23 $\pm$ .04 sec (mean $\pm$ SEM) and 2.6 $\pm$ .6 sec for the fast and slow components, respectively. The ratio of the current contributed by the fast versus the slow components averaged 3.28 $\pm$ .58. Forskolin, an adenylate cyclase activator, increased the rate of desensitization in both cell types. The half-time of desensitization was decreased by 38 $\pm$ 7% (n= 4 cells) in hippocampal neurons and 61 $\pm$ 3% (n= 9 cells) in NG108-15 cells following the application of 30 uM forskolin. This suggests that desensitization of the 5-HT_3 receptor may be regulated by phosphorylation.

369.5

POSTSYNAPTIC MECHANISMS OF ADENOSINE MEDIATED INHIBITION IN CA1 NEURONS, IN VITRO. H.L. Haas*, U. Gerber* & R.W. Greene, Johannes Gutenberg-Universitat, Mainz, FRG; Harvard Med. Sch./VAMC, Brockton, MA 02401

Endogenous adenosine (AD) exerts an inhibitory tone on CA1 neurons even in the absence of synaptic activity. Exposure to exogenous AD evokes a hyperpolarization probably mediated by an increase in potassium conductance. In the present study, records were obtained from CA1 neurons of adult S.D. rats in vitro, employing single electrode sample and hold voltage clamp to analyze the voltage and ionic sensitivity of AD effects. The amplitude and polarity of the AD evoked current varied with extracellular K (3.2, 6.2, 12.5, 25 mM) as predicted by the Nernst equation for a change in potassium permeability. I/V plots (membrane potential was altered from -120 to -50 mV at a rate of 1 mV/200 mS) showed an AD evoked increase in conductance that was voltage insensitive. Neither the addition of 1-5 mM CsCl nor 500 uM 4-AP to the perfusate affected the AD evoked hyperpolarization. However, the addition of 2 mM Ba reversibly blocked the AD response. Transient outward currents were also examined. I_{AHP}, a calcium dependent K current, was increased but I_A was not. We conclude that AD evoked postsynaptic inhibition is mediated by a voltage insensitive increase in steady state potassium conductance and I_{AHP}. The former is similar in its lack of voltage sensitivity and inactivation to a potassium conductance reduced by ACh (analogous to S current described in invertebrates) in CA1 neurons.

369.6

MEMBRANE POTENTIAL FLUCTUATIONS DO NOT CONTRIBUTE TO SHORT-TERM SYNAPTIC PLASTICITY IN *APLYSIA*. Daniel Gardner. Dept. of Physiology, Cornell Univ. Medical College, New York NY 10021.

At identified inhibitory synapses in the *Aplysia* buccal ganglia, both duration and amplitude of synaptic currents are alterable by manipulating presynaptic activity, producing short-term changes in synaptic efficacy. Recent theoretical reports have suggested modifications to Hebbian learning paradigms in which synaptic efficacy would be altered not by neuronal activity, but by changes in the rate of activity. In an attempt to evaluate a variability-sensing learning rule which uses a biophysically plausible mechanism, I advance and test the hypothesis that membrane potential (V_m) fluctuations themselves produce changes in postsynaptic current (PSC) strength. Fluctuations in V_m produced by complex synaptic input might plausibly ease conformational changes of membrane proteins, producing direct or indirect effects on postsynaptic channels. The hypothesis is tested by injecting excess variance (6×10^{-4} V^2 noise over 1-500 Hz range) into identified voltage-clamped postsynaptic neurons, monitoring synaptic efficacy for 60-180 min, and comparing to control synapses sharing a common pre- or postsynaptic element. Different paradigms using three-cell networks evaluate non-specific and associative effects on individual synapses.

NON-ASSOCIATIVE EFFECTS OF NOISE: PSCs were simultaneously recorded at 1 min intervals from two postsynaptic cells innervated by a common presynaptic neuron, with noise injected into one of the two between PSCs. Synaptic current amplitudes and durations varied in parallel in the two followers; normalizing by control values suggests no non-associative effects of noise injection.

ASSOCIATIVE EFFECTS OF NOISE: In a complementary protocol, a postsynaptic neuron was alternately stimulated by two presynaptic cells, with noise injected during PSCs produced by only one of the two inputs. Neither PSC amplitude nor duration was consistently altered by associative pairing with noise.

I conclude that synaptic efficacy is unaffected by 1-2 h of noise simulating complex excitatory and inhibitory synaptic input from other pathways.

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369.7

AN INCREASE IN POSTSYNAPTIC CALCIUM IS SUFFICIENT TO ENHANCE SYNAPTIC TRANSMISSION IN HIPPOCAMPUS. J.A. Kauer, R.C. Malenka, R.S. Zucker, & R.A. Nicoll. UCSF, San Francisco, CA 94143 and Univ. Calif., Berkeley, CA 94720.

Using the hippocampal slice, we have performed two sets of experiments to define the importance of calcium in the induction of long term potentiation (LTP). Intracellular electrodes were filled with the photolabile calcium chelator, Nitr5, loaded with calcium. Photolysis of Nitr5 that elevates calcium to the low micromolar range markedly increased the size of the EPSP in CA1 pyramidal cells for more than 20 minutes. No effect of light exposure was observed either on the extracellularly recorded population EPSP, or in control experiments using electrodes filled with Nitr5 which was not loaded with calcium, demonstrating that the release of calcium was the factor responsible for the potentiation.

Pairing low-frequency stimuli to the presynaptic afferents with depolarization of the postsynaptic cell has been reported to produce LTP. If Ca^{++} entry is necessary for the induction of LTP, one would expect that by holding a cell near the reversal potential for calcium, one could block LTP. Using Cs^{+}-filled electrodes, pairing 10 afferent stimuli with very strong depolarization of the postsynaptic cell produced little or no potentiation of the EPSP; subsequent pairing of 10 identical stimuli with a smaller depolarization produced a large and long-lasting potentiation. Taken together, these experiments demonstrate that calcium is both necessary and sufficient to trigger postsynaptic processes involved in synaptic potentiation.

369.8

NMDA RECEPTOR ACTIVATION POTENTIATES SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS. R.C.Malenka, J.A. Kauer, & R.A. Nicoll. University of California, San Francisco, CA 94143.

To determine whether NMDA application in the absence of presynaptic fiber activity is sufficient to elicit LTP we have examined the effects on synaptic transmission of iontophoretic application of NMDA onto CA1 pyramidal cell dendrites. A large depolarizing dose of NMDA caused an initial depression of the EPSP followed by an enhancement. A small dose of NMDA, which when paired with hyperpolarizing current injection did not cause an enhancement of the EPSP, caused a pronounced potentiation of the EPSP when paired with depolarizing current injection. The NMDA-induced potentiation was occluded shortly after the induction of LTP and could be mimicked by a tetanus suggesting that LTP and NMDA-induced potentiation may activate the same intracellular mechanisms. However, NMDA-induced potentiation differed from LTP in that its average duration was 15-20 minutes.

Activation of the full complement of glutamate receptors also was not sufficient to elicit LTP since application of glutamate alone or quisqualate and NMDA together always elicited a decremental form of potentiation. It was possible to change, in the same cell, a decremental form of potentiation into a long lasting form by increasing stimulus strength during the LTP-inducing stimulus. Increasing stimulus strength would be expected to recruit smaller diameter, higher threshold fibers. From these results, we propose that what previously has been termed LTP consists of two components, a decremental component which can be mimicked by NMDA receptor activation and a long-lasting, non-decremental component which may require stimulation of small diameter, high threshold afferent fibers.

369.9

Differential distribution of acetylcholine receptor mRNA in myotube nuclei. S. Bursztajn, S.A. Berman and W. Gilbert. Bio. Labs. Harvard Univ.,Cambridge, MA. 02138.

Acetylcholine receptor (AChR) genes are differentially regulated during development. AChRs are expressed on muscle cells which are multiucleated and thus represent an interesting system in which to study nuclear activation and inactivation. We have used in situ hybridization with a genomic DNA probe to examine the distribution of AChR α subunit mRNA at the subcellular level. The α subunit genomic clone was obtained from J.P. Changeux. Cells were hybridized with the labelled AChR α subunit,or with labelled actin DNA probe or poly(U)RNA.The hybrids were detected by nuclear track emulsion autoradiography. Silver grains due to AChR α subunit probe showed a striking perinuclear localization. In contrast, myotubes labelled with an actin probe or poly(U) showed a diffuse distribution of silver grains throughout the myotube. Quantitation of myotube nuclei revealed that 71%+4% of nuclei actively express AChR mRNA,and that the other nuclei express the mRNA at low levels or are inactive. Our results show that myotube nuclei are differentially activated for the expression of AChR α subunit mRNA and possibly other synaptic proteins.

369.10

BIPHASIC CHANGES IN ACETYLCHOLINE BINDING TO POST-SYNAPTIC NICOTINIC RECEPTORS BY BARBITURATES, B.A. Dodson, R.L. Ruch*, L.L. Firestone and K.W. Miller*. Dept. of Anaesthesia, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114 USA.

Preliminary data suggest that barbiturates have a biphasic effect on equilibrium ^{3}H-acetylcholine (^{3}H-ACh) binding to Torpedo postsynaptic acetylcholine receptors (AChR). To study this effect in more detail, we examined barbiturate-dependent changes in % AChR desensitized ($\%R_{hi}$). The studies used membranes purified from Torpedo electroplaque and preincubated with DFP. To determine barbiturate-dependent changes in $\%R_{hi}$, ^{3}H-ACh (40-50nM) was added to equilibrated barbiturate-membrane (20-25nM AChR) solutions (pH 7.0). After 5 secs, ^{3}H-ACh binding was determined by filtration (^{3}H-ACh does not itself induce desensitization itself under these conditions).

At lower concentrations, barbiturates decreased $\%R_{hi}$ from control values (25-30% of AChR) in a mass action-like fashion (n_H=1). At higher concentrations, barbiturates increased $\%R_{hi}$ with slopes (n_H)>1. These findings support both a negative allosteric barbiturate-ACh interaction and a more nonspecific interaction, similar to that described for the alcohols and volatile anesthetics. Therefore, biphasic changes in equilibrium ^{3}H-ACh binding appear to represent two different mechanisms of barbiturate actions on the acetylcholine receptor.

369.11

THE DURATION OF GLYCINE ACTION AT MAUTHNER CELL INHIBITORY SYNAPSES IS DETERMINED BY DIFFUSION, NOT NA-DEPENDENT UPTAKE. M. J.Titmus, D.S. Faber, and H.Korn. Dept. Physiol., SUNY at Buffalo, Buffalo, NY ,14214 ,and Dept. Biotechnologies, Pasteur Inst. , Paris.

Recent evidence for facilitatory interactions between adjacent glycinergic synapses on the Mauthner(M-) cell suggested that diffusion, rather than an active uptake or inactivation mechanism, may account for removal of this transmitter from the synaptic cleft. To test this idea we studied the effects of blocking Na- dependent glycine uptake on the kinetics of inhibitory responses evoked by i)stimulating the collateral network, and ii) glycine iontophoresis. 75% of the extracellular Na was replaced by Li or N-methyl glucamine, using in vivo superfusion. A successful exchange was indicated by a reduction in antidromic spike height and membrane depolarization. Removal of Na had no effect on the time course of the decay of evoked synaptic responses recorded in current (n=7) or voltage (n=4) clamp, when they were compared with controls obtained at the same membrane potential. The durations of the longer lasting (1sec vs 10 msec) conductance changes produced by glycine iontophoresis were also unchanged. However, the magnitudes of these responses were enhanced in Li, with the glycine dose-response curves being shifted to the left by about 0.2 to 0.5 log units. This facilitation does not appear to be due to a change in glycine receptor properties, since the Hill coefficient, which averaged about 2, was unaltered by Li in 4 experiments. We conclude that uptake does not influence the duration of glycine action following its synaptic release but may rather act to maintain a low concentration of this transmitter in the synaptic cleft despite its high serum levels. (Supported in part by NS 21848)

PEPTIDES: PHYSIOLOGICAL EFFECTS IV

370.1

SUPPRESSION OF BARORECEPTOR REFLEX RESPONSE BY ENDOGENOUS ANGIOTENSIN III IN THE RAT. J.Y.H. Chan, S.H.H. Chan and C.D. Barnes. Dept. of Pharmacology, National Yang-Ming Med. Coll., Taipei 11221, Taiwan, and Dept. of VCAPP, Washington State Univ., Pullman, WA 99164-6520.

We investigated in the present study the participation of endogenous brain angiotensin III (AIII) in central cardiovascular regulation in pentobarbital sodium anesthetized rats. AIII (100 pmol) promoted an elevation in systemic arterial pressure and a reduction in the baroreceptor reflex (BRR) response. Blocking or enhancing the endogenous AIII activity respectively with its specific antagonist, Ile^7-AIII (100 nmol), or the aminopeptidase inhibitor, bestatin (200 nmol), on the other hand, augmented the loop gain of the same reflex. The suppressive action of AIII on the BRR response was attenuated, and the augmentative effect of Ile^7-AIII was potentiated, however, when these two peptides were administered simultaneously with bestatin. All these events were significantly different from their controls during the first 10-15 min following injection, parallel to the time-course of a discernible action of AIII and Ile^7-AIII on systemic arterial pressure. Furthermore, microiontophoretically applied AIII significantly blunted the responses of neurons in the nucleus tractus solitarius (NTS) to arterial pressure fluctuations. These data suggest that the endogenous AIII may participate in central cardiovascular control by tonically suppressing the BRR response, possibly at the level of the NTS.

370.2

ATTENUATION OF GUANABENZ-INDUCED CARDIOVASCULAR SUPPRESSIVE EFFECTS BY ANGIOTENSIN III IN THE RAT. S.H.H. Chan, J.Y.H. Chan and D.C. Yin*. Institute of Pharmacology, National Yang-Ming Medical College, Taipei 11221, Taiwan, R.O.C.

We evaluated in the present study the interactions between brain neuropeptides and the central catecholaminergic system, using angiotensin III (AIII) and the cardiovascular effect of the α_2-adrenoceptor agonist, guanabenz, as the respective example in rats anesthetized with pentobarbital sodium. Intracerebroventricular (i.c.v.) administration of AIII (100 or 200 pmol) significantly and dose-relatedly attenuated the hypotensive and bradycardiac effects of guanabenz (50 µg/kg, i.v.). Bilateral microinjection of AIII (20 or 40 pmol) to the nucleus reticularis gigantocellularis (NRGC), a medullary site believed to be intimately related to the antihypertensive actions of the aminoguanidine compound, produced similar results. In addition, i.c.v. administered AIII (200 pmol) altered the effects of guanabenz on the arterial pressure-related single-neurons in the NRGC, in a manner that paralleled the blunted vasodepressive and cardioinhibitory actions of the aminoguanidine compound. When applied microiontophoretically, AIII also significantly decreased the responses of NRGC neurons to guanabenz. Based on these findings, it is conceivable that AIII may lessen the antihypertensive efficacy of guanabenz by reducing the responsiveness of neurons in the NRGC to this agent, resulting in the attenuation of its cardiovascular suppressive potency. (Supported in part by NSC-76-0412-B010-23)

370.3

ENHANCED BAROREFLEX SENSITIVITY IN CAPTOPRIL(CAP) TREATED SHR IS DUE TO AN INHIBITION OF BRAIN ANGIOTENSIN II (AII) MECHANISMS. K. H. Berecek and S. WT. Cheng*, Hypertension Program, Univ. of AL. at Birmingham, AL 35294

We examined whether the increase in baroreflex sensitivity previously reported in CAPtreated SHR was due to an inhibition of brain AII mechanisms. Pregnant and lactating female SHR were given oral captopril (100 mg/kg/day). After weaning, pups were maintained on CAP (50 mg/kg) until the study (19-21 wk). Ten days prior to study CAP and control (CON) SHR were given an intracerebroventricular (ICV) infusion of AII (7.5 ng/hr), or vehicle (VEH, artificial CSF). Baroreflex control of heart rate (HR) was assessed in response to changes in mean arterial pressure (MAP) induced by phenylephrine and nitroprusside. ICV AII had no effect on MAP in CAP treated (CAPAII 124±7 vs CAPVEH 119±4) or CON (CONAII 164±7, CONVEH 157±6 mmHg) SHR. ICVAII produced a significant ($p<0.01$) rise in HR (CAPAII 378±13 vs CAPVEH 320±11, CONAII 347±9 vs CONVEH 306±10 bpm) and in water intake and a ($p<0.05$) decrease in the slope of the relationship between pulse interval and MAP but only for CAPSHR (increase in MAP: CAPAII 0.81±.08; CAPVEH 2.8±0.9 CONAII 0.57±0.1, CONVEH 0.67±0.07, decrease in MAP CAPAII 0.46±0.1 CAPVEH 0.85±0.2; CONAII 0.78±0.09, CONVEH 0.99±0.2). Our findings suggest that decreased brain AII activity induced by CAP underlies increased baroreflex sensitivity in SHR.

370.4

THE REGULATION OF OXYTOCIN AND VASOPRESSIN SECRETION BY NEUROHYPOPHYSIAL CO-PEPTIDES. C.A. Bondy* and H. Gainer, LNC, NINCDS, Bethesda, Md. 20892.

Dynorphin, which is co-localized with vasopressin(VP) in the hypothalamo-neurohypophysial system, inhibits the electrically evoked, Ca^{++} dependent secretion of oxytocin(OT) [Bondy et al, Endocrinol.122:1224, 1988]. We have examined the regulatory roles of cholecystokinin(CCK) and corticotropin releasing hormone(CRH) which are co-localized with OT in many magnocellular neurons. Both of these peptides produce significant increases in OT and VP release from rat neurohypophyses *in vitro*.

High affinity CCK receptors were demonstrated autoradiographically in the rat neural lobe(NL). We found that CCK-8 produced a large augmentation of OT and VP secretion from isolated NLs with an EC50 of 10^{-10}M. CCK's stimulatory effect was not dependent upon electrical stimulus or the presence of Ca^{++} in the incubation medium, but was blocked by staurosporine, an inhibitor of C kinase. CRH(500nM) caused a Ca^{++} dependent stimulation of OT and VP secretion from neuro-intermediate lobes(NILs) but had no effect on NLs alone. The application of α-melanocyte stimulating hormone to NLs induced a pattern of OT and VP release identical to that produced by CRH on NILs. Thus, co-peptides released with VP or OT may act locally, either directly on the NL, or via an interaction with the adjacent pars intermedia, to modulate OT and VP release from the nerve terminals of the neurohypophysis.

370.5

INTRATHECAL ARGININE^8-VASOPRESSIN PRODUCES DIFFERENT CARDIOVASCULAR RESPONSES IN CONSCIOUS AND ANESTHESIZED RATS. A. Martinez-Arizala, J. B. Long, and J. W. Holaday. Dept. of Medical Neurosciences, Walter Reed Army Inst. of Research, Washington D.C. 20307-5100.

Prior studies in our laboratories suggested that the cardiovascular changes produced by intrathecal (i.t.) arg^8-vasopressin (AVP) in conscious rats differed from those in anesthesized rats. To further characterize the differences in the responses to i.t. AVP , S-D rats were implanted with PE catheters in the tail artery, the jugular vein, and the lumbar subarachnoid space. Mean arterial pressure (MAP) and hindlimb motor function were monitored following i.t. injections of AVP (0.01 nmoles). Both conscious and anesthesized (ketamine and xylazine) rats had a pressor response to i.t. AVP and conscious rats exhibited an acute hindlimb paralysis. The pressor effect of i.t. AVP were more striking in conscious rats (mean ↑ in ' of 73 ± 3 mmHg) than in anesthesized rats (30 ± 8 mmHg). effects of i.t. AVP were mediated centrally at the V_1 recept since they were blocked by the i.t., but not the i.v. injection c the V_1-receptor antagonist d(CH$_2$)$_5$[TYR(Me)^2]AVP. In addition, the pressor effects of i.t. AVP were: a) blocked by phenoxybenzamine and hexamethonium in the anesthesized, but not in the conscious rats, and b) not blocked in conscious rats by the angiotensin II antagonist [Sar^1, Thr^8]-angiotensin II. These results indicate that sympathetic catecholaminergic mechanisms mediate the rise in MAP produced by i.t. AVP in anesthesized rats, but not in conscious rats.

370.6

OXYTOCIN-INDUCED PENILE ERECTION AND YAWNING: STRUCTURE ACTIVITY STUDIES. A. Argiolas and M.R. Melis. Department of Neurosciences, University of Cagliari, Via Porcell 4, 09100 Cagliari, Italy.

The effect of the i.c.v. injection of several oxytocin (OXY) related peptides on penile erection and yawning was studied in male rats. Substitution of two aminoacids caused marked changes in OXY effect, the rank order of potency being: OXY>Thr^4,Gly^7-OXY>Ser^4,Ile^8-OXY>vasopressin (VP) Similarly, deletion of the C-terminal glycinamide as in OXY 1-8 caused a 99% decrease in OXY potency. In contrast, permanent opening of the disulfide bridge by carboxymethylation completely abolished OXY effect. Inactive were also tocinoic acid (OXY 1-6) and the C-terminal tripeptide Pro-Leu-GlyNH$_2$ (OXY 7-9). Penile erection and yawning induced by OXY-related peptides were antagonized in a dose-dependent manner by potent and selective OXY antagonists such as d(CH$_2$)$_5$Tyr(Me)-Orn^8-vasotocin and (Pen^1,Phe(Me)^2,Thr^4 Orn^8)-OXY and, much less effectively, by the selective VP antagonist d(CH$_2$)$_5$ Tyr(Me)-Arg^8-VP. Carboxymethylated OXY, OXY 1-6 and OXY 7-9 were devoid of antagonistic activity. The present results suggest that central oxytocin receptors mediating the induction of penile erection and yawning are structurally related to those mediating OXY effects in the uterus and mammary gland.

370.7

HYPOTHALAMIC ATRIAL NATRIURETIC PEPTIDE RECEPTORS IN ADRENALECTOMIZED, HYPOPHYSECTOMIZED AND VASOPRESSIN-DEFICIENT RATS. Pero Castrén and Juan M. Saavedra, Laboratory of Clinical Science, National Institute of Mental Health.

Atrial natriuretic peptide (ANP) has receptors in supra-optic and paraventricular nucleus of the hypothalamus; these nuclei are implicated in the regulation of the hormone release from the pituitary gland. We have studied the physiological role of these receptors by measuring ANP binding in hypophysectomized (HX) and adrenalectomized (ADX) rats and in vasopressin-deficient homozygous Brattleboro rats (DI) by receptor autoradiography according to Kurihara et al. (Brain Res. 408:311-39,1987).

Following HX, ANP binding sites were increased in both supraoptic (37±6 and 20±7 mg/fmol protein for HX and controls, respectively, P<0.001) and magnocellular paraventricular nuclei (35±5 and 16±6 fmol/mg protein for HX and control, respectively, P<0.001). A similar increase was observed in DI rats (26±2 and 13±1 fmol/mg protein in SON and 13±1 and 9±1 fmol/mg protein in mPVN for DI and control rats, respectively, P<0.05). ADX did not alter ANP binding in these nuclei.

These results support the hypothesis that hypothalamic ANP receptors are mainly involved in the regulation of vasopressin release.

370.8

INTRATHECAL (I.T.) VASOACTIVE INTESTINAL POLYPEPTIDE (VIP), SUBSTANCE P (SP) AND C-FIBER STIMULATION (CS) HAVE ALTERED EFFECTS AFTER PERIPHERAL NERVE INJURY.

Z. Wiesenfeld-Hallin and R.G. Hallin, Dept. Clin. Neurophysiol., Karolinska Institute, S-141 86 Huddinge, Sweden.

VIP and SP are found in separate populations of primary afferents. After peripheral nerve section the SP content of spinal ganglion cells decreases whereas VIP level increases. Changes in spinal reflex excitability after i.t. VIP, SP and CS were compared in rats with intact or sectioned peripheral nerves.

The hamstring flexor reflex was examined in decerebrate, spinalized, unanesthetized rats. SP and VIP were injected i.t. via a catheter with its tip on the lumbar enlargement. The reflex was evoked by electric shocks (sural n., 0.5ms, 5mA, 1/min), recorded as EMG signals and integrated. In some animals the sciatic n. was sectioned unilaterally 14–21 d. before.

VIP, SP or a brief CS train (20, 1Hz) increased spinal excitability for 2–5 min. SP+CS caused a synergistic facilitation of reflex excitability, which VIP+CS did not. After nerve section SP, VIP and the CS train facilitated the reflex as in intact animals. However, synergism between SP+CS was not observed, whereas VIP+CS had a strong synergistic effect.

SP, VIP and the CS train caused similar reflex facilitation, which may indicate that the CS train releases peptides at afferent central terminals. Synergism between SP+CS train when the sciatic nerve was intact may indicate that other peptides released by the CS (e.g. calcitonin gene-related peptide) interact with SP. No such interaction occurs with VIP. The altered interaction of SP+CS train and VIP+CS train after nerve section may indicate that postinjury changes in peptide levels can be associated with changes in spinal function.

370.9

IONIC BASIS FOR SUBSTANCE P EXCITATION IN TRIGEMINAL ROOT GANGLION (TRG) NEURONS I. Spigelman and E. Puil, Dept. of Pharmacology & Therapeutics, Faculty of Medicine, University of British Columbia, Vancouver, Canada, V6T 1W5.

The slow, depolarizing responses in the perikarya of TRG neurons to substance P applications (Spigelman and Puil, Can. J. Physiol. Pharmacol., 1988) are of special interest because of possible involvement in the transmission of nociceptive impulses. Initially we proposed that substance P excitation in TRG neurons resulted from an increased Na^+-conductance (g_{Na}) in combination with a blockade of a K^+-conductance (g_K). We now provide direct evidence using single electrode voltage clamp techniques for an activation of inward current during applications of substance P (2 µM) in the presence of 4-aminopyridine (1 mM) and tetraethylammonium (10 mM). This current also was observed during internal Cs^+-blockade of K^+-channels. Because changes in g_{Na}/g_K could result from actions on the Na^+/K^+ pump, the effects of extracellular removal of Mg^{2+} were studied on the depolarizations evoked by substance P. The responses were drastically reduced in Mg^{2+}-deficient solutions. The above observations imply that the ionic mechanism of substance P action on TRG neurons is unlike that proposed in central neurons. Also, extracellular [Mg^{2+}] may modulate the substance P responses at the level of peptide-receptor interaction.

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370.10

SYNERGISTIC EFFECTS OF β-PREPROTACHYKININ-DERIVED PEPTIDES ON SALIVARY GLAND SECRETION. Y. Takeda and J.E. Krause, Department of Anatomy & Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110

The preprotachykinin (PPT) precursors encoding Substance P (SP) and Neurokinin A (NKA) display a high degree of homology in regions that do not encode these tachykinins, and the precursors may be differentially processed post-translationally. These observations have led us to examine some biological responses due to these other peptides, and to examine whether they have coordinate actions with SP or NKA on their biological endpoints. We examined the activity of β-PPT derived peptides on salivation responses in rats, and document potent effects of Neuropeptide K [NPK; β-PPT-(72-106)-peptide amide] on the salivation response as well as its synergism with SP in inducing salivary gland secretion. The rank order of potency of femoral vein injected β-PPT-derived peptides on the salivation response was as follows: NPK > SP > NKA, and others, including β-PPT-(72-96)-peptide, were inactive. NPK-induced responses occurred at lower doses than that of SP, and the maximal response was greater. Salivation responses induced by NPK and SP were antagonized by the tachykinin antagonist ([D-Pro^2,D-Trp^{7,9}]-SP), but not by atropine. The potency of NPK (Ki=100 nM) on the displacement of ^{3}H-SP binding to the SP-P type receptor in rat submandibular gland membranes was 100-fold lower than that of SP (Ki=1.00 nM). When both NPK and SP were co-infused for 10 min at submaximal doses, these peptides greatly stimulated salivary secretion over a similar time course than either peptide alone, but the total response was some 3-fold greater than the sum total of individually infused NPK and SP.

We conclude that NPK is a potent tachykinin, and the synergistic effects of NPK and SP on the salivation response may represent an important mechanism of tachykinin cotransmission in tachykinin-elicited biological responses.

370.11

CHRONIC TREATMENT WITH THE POTENT PERIPHERAL CHOLECYSTOKININ ANTAGONIST L-364,718 PRODUCES MULTIPLE PATHOLOGICAL CHANGES IN THE RAT. RB Murphy, LH Schneider, J Sidhu#, JT Pedersen#, MR Pincus#, ES Corp#, SC Weatherford, CA Watson#, J Gibbs and GP Smith. Dept. Psychiatry, NY Hosp-Cornell Med. Ctr., White Plains, NY 10605; Dept. Chemistry and Dept. Pathology, New York Univ., and Manhattan VA Hospital, NY, NY 10032

We treated rats chronically with the potent cholecystokinin (CCK) antagonist L-364,718 for a 14-day period at a dose of 1.5 mg-kg^{-1}-day^{-1} (CA Watson et al, Soc. Neurosci. Abstr., this meeting). In the treated animals (N=9), the spleens were uniformly and grossly enlarged as compared to vehicle-treated controls (N=9) and were distinguished by a diffuse scattering of collections of histiocytes resembling small, non-necrotizing granulomas in the red pulp area. The pancreas in treated animals showed a diminution in the size of the Islets of Langerhans. Other organs, including the exocrine pancreas, were grossly normal. Enlargement of the spleen was not observed in acutely-treated (1-day) rats. Our results strongly suggest that chronic blockade of peripheral CCK receptors with large doses of L-364,718 produces multiple pathological changes. The functional changes associated with these alterations are currently under investigation.

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370.12

EVIDENCE FOR ARTERY-VEIN DIFFERENCES IN RESPONSE TO NEUROPEPTIDE Y IN MESENTERY OF GUINEA PIG. T.L. Anthony* and D.L. Kreulen, Department of Pharmacology, University of Arizona, College of Medicine, Tucson, AZ. 85724.

Neuropeptide Y (NPY) is present in sympathetic nerves that innervate blood vessels. The present study was performed to determine the effects of (NPY) in vascular smooth muscle of the guinea pig mesenteric artery and vein. Membrane potentials were measured with intracellular microelectrodes and contractions were measured on isolated ring segments of the vessels. NPY (65 to 425 pmoles) was administered by pressure ejection from micropipettes placed 95 μm from the tissue surface. NPY evoked a dose-dependent depolarization in mesenteric artery and vein that resembled a slow depolarization not an excitatory junction potential (EJP). The depolarization in vein averaged 8.7 mV and in artery it averaged 2.6 mV. The duration of the depolarizations averaged 10.6 s in artery and 6.2 s in vein. If the NPY was administered at intervals less than 5 min the amplitude of the depolarizations diminished. The amplitude of the EJPs evoked with nerve stimulation was increased 12% (n=2 cells) after pressure ejection of NPY and this effect persisted after the membrane had repolarized. NPY was equipotent for producing contractions in artery and vein in isolated ring preparations. However NPY was more effective in vein than in artery when compared to the norepinephrine maximum response. These experiments demonstrate that NPY could function as an excitatory neurotransmitter in both mesenteric artery and vein to produce a slow depolarization and to potentiate neuromuscular transmission. Support DK36289.

CEREBRAL METABOLISM AND BLOOD FLOW II

371.1

ANIMATING 3D STRUCTURE-FUNCTION RELATIONSHIPS

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Recent progress in neuro imaging has extended analysis of anatomic and physiologic data from 2D to 3D. Research in our laboratory and others have concentrated on techniques for modelling, surface rendering and brain mapping. The result has been the production of accurate and realistic displays of whole brain. However, as the complexity of these models increased, static presentations have become less comprehensible. The problem has been exacerbated by extending the domain to four and five dimensions by introducing functional data, time course and statistical information.

We have addressed this problem by adding movement during the analysis of 3D reconstructions. Real time animation enables the viewer to visualize complex functional anatomic shapes and the spatial relationships between them.

Autoradiographic and histologic data from rats were reconstructed to 3D models by assembling 600 coronal sections per animal. These 3D data sets were used as the basis of an animation that illustrates the process of synthysizing spatially accurate models of structure (histology) and function (autoradiography). Each view (frame) of the 3D brain was computed, displayed and written to video tape. The tape was stopped between views, repositioned and readied for recording the next frame. A frame consisted of 1/30 sec. during tape playback. The results of our efforts are in the form of a video movie.

The movie, which will be shown during the session, illustrates the complex relationships between structure and function during focal seizures in rat. Various forms of rendering and display will be demonstrated.

371.2

STEREOTAXIC COORDINATES AND Z SCORE TRANSFORMATION SIMPLIFY GROUP METABOLIC ACTIVITY DATA ANALYSIS. H.H. Holcomb, H.L. Loats, C.A. Tamminga, J. Links*, R.F. Dannals*, H.N. Wagner* U. Md. Psych. Res. Ctr., Johns Hopkins Medical Institutes, Baltimore, Md., 21205.

Functional image analysis (positron emission tomography [PET] and deoxyglucose autoradiography [2DG-QAR]) is complicated by (1) nonhomogeneous data sets, and (2) inexact, inconsistent anatomical characterization of functional images. These two problems are partially solved by data normalization and statistical transformation (all pixels are Z score converted). Images for a given individual are normalized by dividing each pixel by the whole brain mean gray-plus-white value. Z score transformation is accomplished by subtracting each pixel value from the group mean and dividing that difference value by the sigma associated with that group mean. All functional images, PET and 2DG-QAR, are registered with their anatomical substrates. PET images are registered with integrated 3 mm thickness magnetic resonance images (MRI) and 2DG-QAR with their original tissue sections. 3 Dimensional stereotaxic coordinates are generated from orthogonal, registered MR images, using the bicommissural axis, midline, as the zero point. Image coordinates acquired from planes oblique to the AC-PC line are geometrically transformed. 2DG-QAR coordinates derive directly from stereotaxic apparatus measurements, facilitated by partitioning the brain into one centimeter blocks at known coordinates. Z score activity transformation and 3 dimensional coordinate insertion both facilitate group image analysis.

371.3

REPRODUCIBILITY OF REGIONAL CEREBRAL BLOOD FLOW MEASUREMENTS IN NORMAL SUBJECTS WITH AND WITHOUT AUDITORY STIMULATION . *E. Meyer,* R.J. Zatorre, A.C. Evans,* B. Alivisatos,* and S. Marrett**. McConnell Brain Imaging Unit, Montreal Neurological Institute, McGill University, Montreal, Canada H3A 2B4

Regional cerebral blood flow (rCBF) was measured in six normal, right-handed, young male volunteers using the intravenous $H_2{}^{15}O$ bolus technique and positron emission tomography (PET). On each subject, 5 baseline and 5 stimulation studies were carried out on different days. In both conditions, the subjects' eyes were open. During the stimulation condition, binaural broadband white noise at a sound pressure level of 75 dB was applied via miniature earphones. For each subject, matched magnetic resonance and PET images were collected which allowed precise anatomical localization of the measured rCBF distribution.

Regional CBF across each group of 5 studies was examined for reproducibility and variability. A significant gradual reduction in global CBF across the 5 studies was observed in both conditions (a 17 % drop between the first and last studies). The white noise condition did not reduce the rCBF variability with respect to the baseline condition. It did, however, produce a significant increase in the normalized rCBF value for the primary auditory cortex.

Supported by MRC grant SP-5 and the Killam Scholarship Fund of the Montreal Neurological Institute.

371.4

REGIONAL CEREBRAL METABOLISM OF REM AND NON-REM HUMAN SLEEP AS ASSESSED BY PET J.C. Wu(1), M.S. Buchsbaum(1)*, J.C. Gillin(2), (1)Dept of Psychiatry, UCI, Irvine, CA 92717, (2)Dept of Psychiatry, UCSD

INTRODUCTION: This is the first PET study of sleep with subjects studied under natural nighttime conditions with full polysomnographically recorded sleep. METHOD: Thirty-six normal volunteers were accommodated to the sleep lab. Subjects went to sleep at 2300. 18 FDG was infused after sleep EEG indicated the desired sleep stage. Subjects were roused 32-45 minutes after FDG injection and scanned on a NEUROECAT II scanner (FWHM=7.6mm). Twelve subjects for REM, NREM, and eyes closed awake controls, who met the criteria of spending 75% of their uptake in the desired stage, were studied. RESULTS: NREM sleep showed a significant decrease compared to waking, whereas REM sleep showed an increase (significant sleep stage group effect, $F=5.99$, $d.f=2,3$ $p<.01$). Cortical decreases in NREM were greater than subcortical (basal ganglia, thalamus) decreases. Cortical increases in REM were greater than subcortical changes.

371.5

BRAIN GLUCOSE METABOLISM DURING FASTING IN MAN STUDIED BY POSITRON EMISSION TOMOGRAPHY. *L.J. Hoffer,* C. Redies, C. Beil,* E.B. Marliss,* A.C. Evans,* F. Lariviere,* S. Marrett,* E. Meyer,* M. Diksic,* and A.M. Hakim** (SPON: *G. Karpati*). Montreal Neurological Institute and McGill Nutrition and Food Science Centre, McGill University, Montreal, Canada H3A 2B4

Positron emission tomography (PET) was used to study cerebral glucose and oxygen metabolism, blood flow, and blood volume in four hospitalized obese men (age 38±6SD years, body mass index 36±4 kg/m^2). PET studies were carried out on a control diet (10–14 hours after previous meal), and after 20–24 days of a total fast.

Cerebral metabolic rate for glucose (CMRglc) and glucose tracer rate constants (K_1^* to k_3^*) were measured using ^{18}F labeled fluorodeoxyglucose and dynamic imaging. Cerebral metabolic rate for oxygen, blood flow, blood volume, and oxygen extraction ratio were measured with ^{15}O labeled O_2, CO_2, and CO gases.

Regional CMRglc fell to 38–47% of control values ($p<0.002$). The rate constant for tracer phosphorylation (k_3^*) fell to 42–57% of control values ($p<0.002$). Both parameters decreased relatively uniformly throughout the brain. Regional blood-brain barrier transfer rate constants for tracer (K_1^* and k_2^*), metabolic rate for oxygen, blood flow, and blood volume were unchanged.

Supported by MRC grant SP-5, and fellowships from MRC, DFG (Re 616/1-1), Max-Planck Gesellschaft, and Juvenile Diabetes Foundation.

371.6

SEQUENTIAL DOUBLE-LABELING WITH 2-DEOXYGLUCOSE (2-DG): A TEST OF THE PRIMARY ASSUMPTION. D.L. McEachron, P. Hand, and C.R. Gallistel, Image Processing Center, Drexel Univ., Philadelphia, PA 19110

Double-labeling with 2-DG involves injecting a bolus of 2-DG labeled with [14]C, waiting 45 minutes, injecting a second bolus of 2-DG labeled with another radioisotope and applying a simulus during the second 45 minute period. The relative contributions from the different radioisotopes are separated using different films, when using [3]H, or utilizing the shorter half-life of [18]F. The idea behind the technique is to use each animal as its own control. The primary assumption is that original [14]C-labeled 2-DG does not relocate in response to stimulation during the second labeling period.

Preliminary results of the following experiments were reported in Science, Vol. 238 as a technical comment (McEachron, et.al. : 1586, 1988). Eight rats were prepared for self-stimulation (Gallistel, et.al., J. Neurosci. 5: 1246, 1985) and given [14]C-2-DG (80 μCi/rat) in an intraperitoneal injection. Four rats were stimulated from 0-45 minutes post-injection, 2 of which were sacrificed at 45 minutes and 2 at 90 minutes post-injection. Four animals were stimulated from 45-90 minutes post-injection and sacrificed at 90 minutes. Responses to stimulation in the ventral limb of the Diagonal Band of Broca and right posterior MFB were similar in all animals stimulated from 0-45 minutes and in 2 of four animals stimulated from 45-90 minutes.

To further assess the stability of labeled 2-DG during stimulation from 45-90 minutes post-injection, young male rats were prepared for full quantification procedures (Sokoloff, et.al., J. Neurochem. 28: 897, 1977). At time 0, 50 μCi of [14]C-2-DG was given intravenously. From 0-45 minutes post-injection, the left C3 was stimulated by tactile whisker stimulation while from 45-90 minutes, the right C3 was stimulated in exactly the same manner. Blood was collected during the entire 90 minute procedure and the autoradiograms were analyzed for local cerebral glucose utilization (LCGU) using the DUMAS imaging system.Comparison of the left and right cerebral cortices in 5 rats indicated that between 39-68% of the increase in LGCU over background levels recorded in the first 45 minutes occurred during the second period (from 45-90 minutes) This confirms the results from the self-stimulation experiment and suggests that the primary assumption in sequentail double-labeling is invalid.

371.7

DOUBLE-LABEL DEOXYGLUCOSE METHOD APPLIED TO FERRET VISUAL CORTEX. *C. Redies and M. Diksic**. Montreal Neurological Institute, McGill University, Montreal, Canada H3A 2B4

The quantitative double-label deoxyglucose method (Redies et al., Neurosci.22:601,1987) allows the separation of glucose utilization patterns in mammalian brain elicited by two sequential sensory stimulations.

The method requires the knowledge of the rate constants for transfer of tracer across the blood-brain barrier (K_1^* and k_2^*), tracer phosphorylation (k_3^*), and loss of metabolized tracer (k_4^*), and of the lumped constant (LC). These constants were determined for fluorodeoxyglucose (FDG) and 2-deoxyglucose (2-DG) in 18 ferrets killed between 2.5 and 180 min after tracer injection. Mean gray matter values for [^{14}C]2-DG are: $K_1^* = 0.19$ml/g/min, $k_2^* = 0.28$/min, and $k_3^* = 0.093$/min; and for [^{3}H]FDG: $K_1^* = 0.21$ml/g/min, $k_2^* = 0.29$/min, and $k_3^* = 0.15$/min. Results suggest that loss of metabolized tracer (k_4^*) occurs at a constant rate of 0.01/min for 180 min after injection. However, whether k_4^* is 0 or 0.01/min, has a negligible effect on calculating glucose utilization in conventional 45 min 2-DG experiments provided that the entire analysis including the determination of the LC is consistent. Assuming $k_4^* = 0.01$/min, the LC in ferret brain is 1.03±0.08SEM for FDG and 0.70±0.09 for 2-DG.

Double-label experiments in the ferret visual cortex show that the method provides layer-specific information on functional "columns" elicited by two different sequential visual stimulations in the same animal.

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371.8

COMPARISON OF METABOLIC TRAPPING OF RADIOACTIVITY FROM (^{14}C)-2-DEOXYGLUCOSE AND (6-^{14}C)-GLUCOSE IN RAT BRAIN. W.E. Stumpf, G.E. Duncan* and G.R. Breese. Dept. Cell Biol. and Anat. Univ. of N.C., Chapel Hill, NC 27599.

The uptake and retention of radioactivity was measured in areas of rat brain at different times after i.v. injection of (^{14}C)-2-deoxyglucose (2-DG) or (6-^{14}C)-glucose (G) with nuclear emulsion autoradiography. In most brain regions the accumulation of radioactivity from the two compounds was similar using a 30 min survival period for G and a 45 min survival period for 2-DG. However, at those times, relatively more radioactivity accumulated from 2-DG in stratum lacunosum-moleculare of hippocampus and layer 4 of isocortex. In contrast, relatively more radioactivity accumulated from G in the dentate gyrus, CA-1 pyramidal cell layer of hippocampus, and layer 2 of piriform cortex. With G at 5 and 30 min survival periods, the distribution of radioactivity was identical except in layer 4 of isocortex, where more radioactivity was present at 5 min. With 2-DG at 5 and 45 min survival periods the relative and absolute amount of radioactivity was greater at 5 min compared to 45 min in the dentate gyrus, CA-1 pyramidal cell layer, and in layer 2 of piriform cortex. In other brain regions, the absolute and relative amount of radioactivity was similar or slightly greater at 45 min compared to 5 min. These results demonstrate that neuroanatomically selective loss of radioactivity occurs after injection of 2-DG during a 45 min survival period.

371.9

CENTRAL ACTION OF PSYCHOMOTOR STIMULANTS ON LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) IN EXTRAPYRAMIDAL MOTOR AREAS. A.S. Kimes, G. Wilkerson*, C. Ori* and E.D. London. Neuropharmacol. Lab., NIDA Addiction Res. Ctr., Baltimore, MD 21224.

Psychomotor stimulants, such as l-cocaine, ±3,4-methylenedioxymethamphetamine (MDMA), d-amphetamine and phencyclidine (PCP), produce stereotyped behaviors and increase LCGU in the extrapyramidal motor system as well as other brain areas of rats. It seemed possible that LCGU effects in motor areas could be secondary to increased limb movements rather than due to primary actions in the brain. We, therefore, used the 2-deoxy-D-[1-^{14}C]glucose method to measure LCGU in paralyzed (gallamine, 60-80 mg/kg, i.v.) and artificially ventilated rats treated with the aforementioned drugs. Rats received i.p. injections of saline, cocaine (30 mg/kg) or MDMA (30 mg/kg); or i.v. injections of saline, PCP (5 mg/kg) or amphetamine (5 mg/kg) 5 min before radiotracer injection. Cocaine and MDMA stimulated LCGU in the globus pallidus, substantia nigra pars reticulata and cerebellum. Amphetamine elevated LCGU in the same areas and also the anteroventral thalamus, zona incerta, subthalamic nucleus (n.) and red n. PCP elevated LCGU in the red n., substantia nigra pars reticulata, subthalamic n., zona incerta, anteroventral thalamus, globus pallidus and caudate putamen. As these effects were seen in paralyzed rats, they were not secondary to limb movements, but rather were primary central effects.

371.10

GLUCOSE UTILIZATION IN DISCRETE BRAIN AREAS AFTER CHRONIC ADMINISTRATION OF IMINODIPROPIONITRILE IN RATS. A. Della Puppa*, J.L. Cadet and E.D. London (SPON: J. Johnson). Neuropharmacology Lab., NIDA-ARC, Baltimore, MD 21224; Dept. of Neurol. Columbia Univ., New York, NY 10032.

The chronic administration of the neurotoxin iminodipropionitrile (IDPN) to rats causes a persistent behavioral syndrome consisting of random circling, vertical head twitches and hyperactivity (Chou, S.M., Acta. Neuropath., 3:428, 1964). The 2-deoxy-D-[1-^{14}C]-glucose method was used to assay the local cerebral glucose utilization (LCGU) in discrete brain areas.

Twelve male Sprague-Dawley rats were assigned to one of two groups, receiving a single daily i.p. injection of saline or IDPN, respectively. One day after the development of the characteristic behavioral syndrome, LCGU was measured as previously described (Sokoloff et al., J. Neurochem., 2:897, 1977). Statistically significant decreases in LCGU were found within the superficial and deep layers of the superior colliculus, interpeduncular nucleus, inferior colliculus, medial and lateral geniculate nuclei and medial and lateral vestibular nuclei. Nonsignificant decrements were seen in the substantia nigra. The claustrum showed a 34% increase in LCGU.

These findings suggest that IDPN affects the nigrotectal pathways controlling head and neck movements via reticulospinal tracts. This effect may also contribute to the phenomena of IDPN-induced, persistent spasmodic dyskinesias in rats.

371.11

ACUTE COCAINE DECREASES REGIONAL CEREBRAL GLUCOSE UTILIZATION IN HUMAN SUBSTANCE ABUSERS. E.D. London, N.G. Cascella*, D.F. Wong*, M. Sano, R.F. Dannals*, J. Links*, R.I. Herning*, J.K.T. Toung*, H.N. Wagner, Jr.* and J.H. Jaffe*. Neuropharmacol. Lab. NIDA Addiction Res. Ctr. and Johns Hopkins Univ., Balto., MD.

Regional cerebral metabolic rates for glucose (rCMR-glu) provide indices of brain function under various conditions. To clarify mechanisms mediating cocaine euphoria, we initiated a double-blind, placebo-controlled, crossover study of cocaine (C) effects on rCMRglu by the PET [^{18}F]fluorodeoxyglucose (FDG) method. Subjects for the study were men, 23-38 years of age, with a history of polydrug abuse. Self reports and EEG responses to 20 or 40 mg C or placebo (P), i.v., recorded on 4 separate days before the PET study, indicated that C increased EEG *beta* activity and produced euphoria. The subjects underwent 2 FDG scans, with 40 mg C or P given simultaneously with FDG. Rates of rCMRglu were measured in 22 regions of interest. C generally reduced mean rCMRglu, particularly in parts of the parietal and occipital cortices ($\geq$ 26% of P values). Decrements in most regions were 10-19% of P. Some areas showed minimal or no C-induced changes (cerebellum, insula, lateral thalamus). The observed decreases in rCMRglu suggest that 40 mg C, i.v., selectively reduces cerebral oxidative metabolism and that C-induced euphoria is mediated by a reduction in cortical activity.

371.12

VISIBLE LIGHT AFFECTS ^{14}C-2-DEOXYGLUCOSE UPTAKE INTO RAT CEREBRAL CORTICAL TISSUE IN VITRO. P.D. Wade$^{+\ddagger}$ and P. Siekevitz^{+}, $^{+}$Rockefeller Univ., New York, NY 10021 and ‡New York College of Podiatric Medicine, NY, NY 10035

Prompted by an observation that visible light influences K^+-induced release of GABA from cortical slices, (Soc.Neurosci.Absts.11:1129,1985), we have begun to examine the generality of response to light by measuring its effect on ^{14}C-2-DG uptake into the slices. One-half mm thick cortical slices were incubated with 0.7 µM ^{14}C-2-DG in Ringer's in a dark room in a water bath at 36-37 C for 30 min. either with or without light (1.9 mW/cm^2, broad spectrum, much of the UV removed by glass). The tissue was then rinsed repeatedly with Ringer's until excess radioactivity was removed, and the acid-soluble radioactivity taken up into the tissue was measured. Normalized ratios of uptake in a light expt. to uptake in a dark expt. yielded a mean of 0.89$\pm$0.06 (SEM, n=11). Thus at the one intensity of light used, visible light caused about 10% inhibition of ^{14}C-2-DG uptake into the tissue. As a first approximation, this degree of inhibition might be considered to be in a functional range, since in some in vivo states, functional activity changes have been associated with 10% changes in 2-DG uptake into certain brain regions [Soc.Neurosci.Absts. 11:813 (Nos. 228.9, 228.10),1987]. The intensity of light causing this partial suppression of uptake was the same intensity that caused suppression of K^+-induced release of GABA previously.

MEMBRANE COMPOSITION AND CELL SURFACE MACROMOLECULES I

372.1

GELASMIN, A MAMMALIAN ACETYLCHOLINE RECEPTOR (ACHR) CLUSTERING FACTOR, AFFECTS ACHR SUBUNIT mRNA LEVELS IN EMBRYONIC RAT MYOTUBES: THE EFFECT IS SYNERGISTIC WITH THAT OF CALCITONIN-GENE-RELATED PEPTIDE (CGRP). K.F. Barald. Dept. Anatomy and Cell Biology, University of Michigan Medical School, Ann Arbor, MI 48109.

Gelasmin is a 93kD glycoprotein component of muscle basal lamina (BL) that is found on embryonic rat myotubes prior to innervation. It is not present in motoneurons at any time. After innervation of embryonic myotubes or in adult muscle fibers, gelasmin becomes localized to the synaptic BL. Unlike the Torpedo AChR clustering factor, agrin, gelasmin affects AChR protein levels and localization and AChR α-subunit mRNA levels. Both gelasmin and CGRP increased total receptor protein content significantly: CGRP, as reported previously by Fontaine et al (J. Cell Biol.105,1987), caused a 30% increase in specific ^{125}I-α-bungarotoxin bound; gelasmin a 35-40% increase. While neither CGRP nor gelasmin affected actin mRNA levels, each had a significant effect on AChR α-subunit mRNA levels, measured in Northern blots with a cRNA probe for mouse AChR α-subunit mRNA kindly provided by Dr. Jim Patrick. 15 ug/ml purified Gelasmin induced a 4-5 fold increase in α-AChR mRNA levels; 10^{-7} M CGRP, a 3-4 fold increase (as previously reported by Fontaine et al., 1987) Together, they effected a 6-14 fold increase in AChR α-subunit mRNA levels. The effect of gelasmin and CGRP appears to be synergistic in these preliminary studies. Further studies are underway to determine whether this represents stabilization or induction of the mRNA. Supported by the NSF, NIH, MDA, Phoenix Foundation and Office of the Vicepresident for Research, U. Michigan

372.2

A UNIQUE GLYCOSYLATED FORM OF NEURAL CELL ADHESION MOLECULE PRESENT IN FROG AND RAT PRIMARY OLFACTORY NEURONS. B. Key* and R.A. Akeson. Basic Research, Children's Hospital Medical Center, Cincinnati, Ohio 45229

The neural cell adhesion molecule (NCAM) consists of three isoforms of approximate molecular weights 180, 140 and 120kD in the adult vertebrate nervous system. In the present study we have identified and characterized a new subtype of the high molecular weight form of NCAM present only in the olfactory bulb.

Among a group of new monoclonal antibodies (Mabs) generated against both frog and rat olfactory epithelia were three Mabs (Mabs 5FOE, 9OE and 13FOE) all of which strongly labelled the surface of frog primary olfactory neurons. In frog brain sections Mabs 5FOE and 9OE labelled only the olfactory glomeruli in the main and accessory olfactory bulbs whereas 13FOE labelled cells and axons throughout the whole brain. 9OE cross reacted with rat and labelled glomeruli in only the accessory olfactory bulb. A similar distribution of binding was previously described (Key, B. and Giorgi, P.P., Neurosci. Lett. 69: 131, 1986) for the lectin soybean agglutinin (SBA). In immunoblots all three Mabs recognized a single band (approximately 200kD molecular weight) that was immunoprecipitated by a polyclonal anti-NCAM antibody. SBA binding molecules were isolated by affinity chromatography and shown to be a single band at 200kD which in immunoblots reacted with all three Mabs.

In summary, we have identified a unique glycosylated form of NCAM recognized by SBA and Mab9OE which is restricted to only olfactory neurons in frog and rat. The presence of specific cellular or regional NCAM subtypes may represent a mechanism for modulation of cell-cell interactions. Supported by NIH Grants NS23348 and HD21065.

372.3

ANALYSIS OF CD4 RNA AND PROTEIN IN THE DEVELOPING HUMAN NERVOUS SYSTEM. C.A. Kunsch*, H.T. Hartle* and B. Wigdahl. Department of Microbiology, The Pennsylvania State Univ. Col. of Med., Hershey, PA 17033.

The CD4 molecule functions as a cell surface recognition protein in the immune system and is also found in the nervous system of adults, where its function is currently under investigation. Furthermore, CD4 has been identified as the cellular receptor the human immunodeficiency virus type-1 (HIV-1) and its presence in the nervous system may be involved in the susceptibility of selected neural cell populations to HIV-1 infection. We are currently in the process of defining (i) the presence and distribution of CD4 RNA and protein in tissue and primary cell cultures derived from human fetal central and peripheral nervous system and (ii) the role of CD4 in HIV-1 infection of these cell types. We have shown that neural cells derived from human fetal dorsal root ganglia (DRG) are susceptible to HIV-1 infection and that treatment of these cells with monoclonal antibody directed against the T4A epitope (the HIV-1 binding epitope) but not the T4 epitope resulted in approximately a 60% reduction in HIV-1-specific gag antigen expression. However, we have been unable to detect the physical presence on the cell surface (via fluorescence-activated flow cytometric analysis) or the synthesis of (via immunoprecipitation and gel electrophoresis) the CD4 molecule in neural cell populations derived from DRG. RNA blot hybridization analysis of total cellular RNA isolated from primary human fetal DRG and spinal cord utilizing a [^{32}P]-labeled single-stranded antisense RNA probe demonstrated specific hybridization to two unique species of RNA that migrate slower than 28S ribosomal RNA. However, the 3.0-kb transcript commonly found in CD4$^+$ lymphocytes was not detected. The two unique high molecular weight RNA species were not detected in RNA isolated from human fetal heart or when a radiolabeled sense strand was utilized as probe. The cell type specificity, tissue distribution, and structure of these two unique RNA species, as well as their potential to encode a functional CD4 or CD4-like protein are being examined.

372.4

ANALYSIS OF PROTEOGLYCANS IN THE DEVELOPING RAT CENTRAL NERVOUS SYSTEM A. D. Lander[1] and W. D. Matthew[2] [1]Dept. of Brain and Cognitive Sciences, Mass. Institute of Technology, Cambridge, MA 02139 and [2]Dept. of Neurobiology, Harvard Medical School, Boston MA 02115

Cell culture and immunochemical studies have led to the identification of cell-surface and extracellular proteins likely to play roles in neural cell adhesion, migration and axon navigation. Of these molecules, surprisingly many--including laminin, fibronectin, thrombospondin, tenascin, N-CAM, acidic and basic fibroblast growth factor, retinal purpurin, and glia-derived protease nexin-1--exhibit specific binding to proteoglycans (PGs). PGs are glycoproteins found on cell surfaces and in extracellular matrices, and appear to be involved in the adhesion, migration and proliferation of many non-neural cells.

To identify functions PGs may have in neural development, we have exposed neuronal cultures to glycosaminoglycans (GAGs), the carbohydrate portion of PGs. Micromolar amounts of heparin, for example, but not chondroitin sulfate, inhibit the neurite outgrowth-promoting activity of laminin. Since GAGs could have non-specific effects, and commercially obtained GAGs may differ from those of PGs in the developing nervous system, a demonstration that a molecule such as laminin interacts functionally with neural PGs requires identifying a neural PG to which it binds, and showing that the molecule's *in vitro* effects can be perturbed by an agent (*e.g.* an antibody) specific for that PG. We are therefore fractionating adult and newborn rat brain, enriching for PGs by a single-column procedure, and analyzing them by SDS-gel electrophoresis after treatment with GAG-degrading enzymes. In adult brain, a large chondroitin sulfate PG (core protein M$_r$ ~400 kd) has been found, as well as minor species. A similar large PG is detected in neonatal brain. A monoclonal antibody (MAb) that recognizes this PG has been obtained, and reacts with sections of adult and neonatal brain. Heparan sulfate PGs have also been identified, including two species associated with neonatal brain membranes. Currently, we are examining binding of identified PGs to laminin, fibronectin, and other molecules, as well as continuing to screen MAbs. Supported by the Whitaker Health Sciences Fund (ADL) and NIH NS02253 (WDM).

372.5

MONOCLONAL ANTIBODY IDENTIFIES A PROTEOGLYCAN EXPRESSED BY A SUBCLASS OF GLIAL CELLS. A. Faissner* (SPON: M. Schachner). Dept. of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 364, 6900 Heidelberg, Germany (FRG).

A monoclonal antibody designated 473 was obtained from rats immunized with L2/HNK-1 positive glycoprotein fractions from adult mouse brain. In immunofluorescence double labeling experiments performed on cerebellar cultures of varying ages no overlap with neuronal markers was observed. The antibody stained subsets of immature astrocytes and oligodendrocytes. At earlier stages of culture about half of the 473+ cells did not express any of the conventional markers. Metabolic labeling experiments with subsequent immunoprecipitation performed with glial monolayer cultures in vitro show that the 473 antigen incorporates sulfate, fucose and methionine. It migrates at 10^6 apparent M_r under non-reducing conditions and was recovered from detergent lysates and supernatants of labeled cultures. Material with similar properties could be demonstrated in G26-20 glial tumor cells. Digestion of immunoprecipitates obtained with 473 from metabolically labeled G26-20 cell line supernatants with chondroitinases ABC and AC demonstrates that the 473 antigen is a chondroitinsulfate proteoglycan. The 473 antigen carries the L2/HNK-1 epitope, a carbohydrate common to several adhesion molecules. Functional properties of the 473 proteoglycan are currently being investigated.

372.6

ANALYSIS OF LECTIN BINDING TO DENERVATED AND REGENERATING SKELETAL MUSCLE. G.P. Cole*, S. Iyer*, E.W. Perry* and A.K. Gulati* (SPON: G.S. Doetsch). Section of Neurosurgery and Department of Anatomy, Medical College of Georgia, Augusta, GA 30912.

Ten different fluoroscein-conjugated lectins of various sugar binding affinities were used to compare glycoconjugates in denervated and regenerating rat skeletal muscle. Muscles were denervated by cutting and ligating the sciatic nerve. Muscle regeneration was induced by autotransplanting the extensor digitorum longus (EDL) muscle. Some EDL muscles were reautotransplanted to initiate a second wave of regeneration. Frozen-sections were prepared from all muscles and stained with each lectin. Lectin binding increased with denervation due to the thickening of endomysium. In normal muscle the binding of Glycine max and Bachinia purpurea agglutinins was seen in discrete regions of the endomysium, however, after denervation the distribution became more extensive. Wheat germ agglutinin specifically stained the myogenic zone of the regenerating muscle. Furthermore, in reautotransplanted muscles, Wheat germ agglutinin binding appeared earlier than in muscles transplanted once. These results show that specific changes in glycoconjugates occur during denervation and regeneration.

372.7

MONOCLONAL ANTIBODIES AGAINST NGF-DIFFERENTIATED PC-12 CELL SURFACE ANTIGENS RECOGNIZE CULTURED HIPPOCAMPAL NEURONS. Y. Kuroda, Y. Ohguchi* and K. Kobayashi*. Dept.of Neurochem.,Tokyo Metropol. Inst.for Neurosciences, Fuchu, Tokyo 183, JAPAN.

A library of monoclonal antibodies against cell surface antigens of NGF-differntiated PC-12 cells was obtained. Myeloma was fused with spleen cells of mice injected intact PC-12 cells which were cultured with NGF for 6-8 days. Culture fluid of the resulting hybridoma was screened by ELISA using the intact cells and Millititer filtration system. Many monoclonal antibodies in the library recognized cell surface antigens which expressed on the PC-12 cells in different manner during NGF-treatment.

Hippocampal tissue from 16 days fetal rat were dissociated with trypsin-treatment and cultured for 1-2 weeks. The culture was immunostained with the monoclonal antibodies. Several monoclonal antibodies recognized the cell surface antigens of the cultured neurons which were identified with antibodies against neurofilaments. Some antibodies seemed to recognize extracellular matrix and glial cells. The monoclonal antibody library is an useful tool to study cell surface molecules and their functions not only in PC-12 cells but also in CNS neurons to differentiate.

372.8

ECTO-PROTEIN KINASE AT THE SURFACE OF CNS NEURONS. Y.H. Ehrlich, I. Galbraith*, J. Jackman* and E. Kornecki. Dept. of Psychiatry, University of VT and The Ctr. Dev. Neurosci., CUNY/CSI, Staten Island, NY 10301.

To demonstrate secretion of ATP and ecto-protein kinase activity in the CNS, we have utilized embryonic neostriatal neurons differentiated during maintenance for 15-21 days-in-vitro (DIV) in a chemically defined medium. Depolarization by 50mM KCl induced a Ca^{++}-dependent release of ATP, and stimulation by 100μM veratridine induced ATP secretion that was blocked by tetrodotoxin. Incubation of intact, attached neurons with [γ-^{32}P]-ATP (added to the medium) resulted in radiolabeling of surface phosphoproteins within 10-15 min. These proteins were not phosphorylated when intracellular ATP pools were labeled with equivalent amount of inorganic ^{32}Pi. Phosphorylation of surface proteins with M.W.>50Kd was dependent on 1mM Mg^{2+} but not on Ca^{2+}, whereas phosphorylation of surface proteins with M.W. of 39-42 Kd required 1mM Ca^{2+} and was independent of Mg^{2+}, as we reported previously with synaptosomes (J.Neurochem., 50: 263, 1988). Phosphorylation by extracellular AT^{32}P of proteins co-migrating with N-CAMS was high during the period of rapid neurite extension (4-6 DIV) and declined after synaptogenesis and maturation (15-18 DIV); indicating that ecto-protein kinase activity in the CNS is developmentally regulated. Supported by AFOSR grant no. 88-0004.

372.9

BRAIN SPECTRIN(240/235A): A NOVEL ASTROCYTE SPECIFIC SPECTRIN ISOFORM. L.L. Lopresti*, B.M. Riederer, I.S. Zagon, L.A. Casoria* and S.R. Goodman*, The Cell and Molecular Biology Center, The Milton S. Hershey Medical Center, Hershey, PA 17033

We have previously demonstrated the existence of two distinct isoforms of spectrin in mammalian brain. Brain spectrin(240/235) found in neuronal axons and presynaptic terminals, and brain spectrin(240/235E) located in neuronal cell bodies, dendrites, and postsynaptic terminals (Riederer et al. 1986, J. Cell Biol. 102: 2088). In this study we have prepared a panel of monoclonal antibodies which all react exclusively with the 240kD or 235kD subunits of brain spectrin on Western blots of total rat brain protein. Immunohistochemical analysis of rat cerebellum utilizing 32 distinct monoclonal antibodies, yielded 13 antibodies which gave a staining pattern typical of brain spectrin(240/235E), 3 antibodies which detected brain spectrin(240/235), 1 antibody which detected both isoforms, and 15 antibodies which detected a novel isoform in astrocytes. In rat cerebellum this novel isoform was localized in the soma and fibrous processes of astrocytes and in the distal processes of Bergmann glia, but was not present in oligodendrocytes. The immunoprecipitation of rat brain spectrin with the astrocyte specific monoclonals under stringent conditions, yielded 240k and 235kD subunits in a 1:1 (mol/mol) ratio. We refer to this newly discovered mammalian brain spectrin isoform as brain spectrin(240/235A).

372.10

SEX DIFFERENCES IN 67-COPPER UPTAKE BY HYPOTHALAMIC (HT) AND HIPPOCAMPAL (HIP) SLICES. D.E.Hartter, K.R.Bhasker*, G.Cho* and A.Barnea. Depts. OB/GYN & Physiol, Univ. Tx. Southwestern Med. Center, Dallas, TX. 75235, U.S.A.

Cu is an essential trace metal that is highly concentrated in the brain, particularly in axonal terminals and secretory vesicles. A role for Cu in modulating neuronal function is supported by our previous findings that Cu markedly amplifies prostaglandin E_2 stimulation of peptide release from HT tissue and that newly taken-up 67-Cu is released by depolarization. We have recently demonstrated that HT slices take-up 67-Cu by a saturable, ligand-specific process having an apparent Km ($\approx$40 μM) in the operative conc. range of Cu. We addressed the question: Are there sex and/or regional differences in 67-Cu uptake in the brain? Saturation curves were established for 67-Cu uptake by HT and HIP slices obtained from rat brains. MALES: Vmax for 67-Cu was greater ($P<0.001$) in the HIP than in the HT (650 vs 425 pmol/min/mg P) of intact males and castration (14 days) led to an increase ($P<0.001$) in Vmax in both regions (850 vs 525 pmol/min/mg P). FEMALES: Vmax was similar in HIP and HT of pregnant females (650 vs 700 pmol/min/mg P) and it was reduced ($P<0.001$) by ovariectomy (19 days) only in the HT. There was no difference in the apparent Km in these tissues. Thus, there are sex and regional differences in number of Cu-carrier sites in the HT and HIP and gonadal steroids appear to modulate the process of Cu uptake in the brain.

372.11

GLUCOCORTICOIDS DECREASE GLUCOSE TRANSPORT IN CULTURED HIPPOCAMPAL NEURONS. Heidi C. Horner* and Robert M. Sapolsky, Biology Department, Stanford University, Stanford, California, U.S.A. 94305

Glucocorticoids (GC's) compromise the ability of hippocampal neurons to survive various neurotoxins both in vivo and in vitro. A general effect of GC's in peripheral tissue is inhibition of glucose transport. We have suggested that the classic, catabolic effects of GC's in peripheral tissue may also occur in the hippocampus and may underlie the GC-enhanced damage. The effect of GC's on glucose transport into cultured hippocampal neurons was examined. Transport of ^{14}C-2-deoxyglucose (2-DG) in cells grown in 48-well cluster dishes is linear for 15 mins ($t_{1/2}$= 6.3 mins) and is D-glucose and cytochalasin B inhibitable. Corticosterone (CORT) and dexamethasone (DEX) treatment resulted in a highly consistent and significant 18% ($p<0.001$) and 20% ($p<0.01$) decrease, respectively, in 2-DG transport. The inhibition is dose- and time-dependent becoming maximal at 1 uM CORT and 100 nM DEX after 24 hrs, and half maximal at 60 nM and 7 nM, respectively. Aldosterone inhibited 2-DG transport only at 1 uM, while testosterone, estradiol and progesterone had no effect on 2-DG transport after 24 hrs at concentrations ranging from 1 nM to 1 uM. CORT had no effect on 2-DG transport into neuronal cultures derived from the cortex, cerebellum, midbrain or hypothalamus. Supported by NIH grant AG-06633.

372.12

CALCIOSOME(S) IN MAMMALIAN BRAIN? P. Volpe*, B.H. Alderson*, C.A. Dettbarn*, P. Palade*, B. Bruno*@, and J. Meldolesi@ (SPON: M.C. Andersen). Dept. of Physiology & Biophysics, UTMB, Galveston, TX 77550 and @S. Raffaele Scientific Inst., Univ. Milano, Milano, Italy.

Recent immunocytochemical and biochemical results have provided direct evidence for the existence of a hitherto unrecognized organelle in nonmuscle cells, homologous to the sarcoplasmic reticulum (SR) of striated muscles, which seems to be the inositol 1,4,5-trisphosphate (IP_3)-sensitive Ca^{2+} store and has been named "calciosome" (Volpe, P., et al., Proc. Natl. Acad. Sci., 85:1091-1095, 1988). In the liver and exocrine pancreas, as well as in two cell lines, HL60 and PC12, proteins related to calsequestrin(s) (CS) and Ca^{2+}-ATPase(s), were shown by immunogold labeling to reside not in the endoplasmic reticulum or other previously identified cytoplasmic organelle, but in a population of vesicles and small vacuoles distributed throughout the cytoplasm. We now have data indicating that subcellular fractionation of canine brain yields a membrane fraction that actively accumulates Ca^{2+}, binds (^{3}H)IP_3, releases Ca^{2+} upon addition of IP_3, and contains a protein immunologically related to CS. Immunocytochemistry of cultured neurons indicates that CS-positive organelles are localized both in the soma and neurites. The calciosome is therefore likely to exist in nerve cells. (Supported by NIH grant GM 40068-01).

LIMBIC SYSTEM II

373.1

THE DISTRIBUTION OF PROJECTIONS FROM THE ANTERIOR THALAMIC NUCLEI TO THE SUBICULAR CORTEX. Th. van Groen* and J.M. Wyss (SPON: J.W. Brown). Dept. of Pharmacology, Univ. of Edinburgh, Edinburgh, Scotland and Dept. of Cell Biol. and Anat., Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Small (<20nl) injections of [^{3}H] amino acids or iontophoretic deposition of Phaseolus vulgaris leucagglutinin (PHA-L) were employed to characterize the distribution and termination of anterior thalamic nuclei projections to subicular cortex. Injections into the anterior dorsal nucleus (AD) labeled terminals in the retrosplenial granular cortex (Rga and Rgb), the postsubiculum (POST), the presubiculum (PRE) and the parasubiculum (PARA) but not the subiculum (SUB). In contrast, injections in the anterior ventral (AV) nucleus labeled terminal fields in Rga and b and all areas of the subicular cortex except PARA. Injections into the lateral dorsal nucleus (LD) labeled terminals in the retrosplenial cortex and all areas of the subicular cortex except SUB. The laminar pattern of terminal labeling was:

	Rgb	Rga	Post	Pre	Para	SUB
AD	I,III,IV	I,II	I,III	(I,III)	IV-VI	-
AV	I,IV	I	I	I,III	-	I,II
LD	-	I	I,III	I,III,IV	IV,V	-

These results demonstrate that AD,AV and LD each have unique interconnections with the limbic cortex, and thus, likely have distinct influences on memory.

373.2

TOPOGRAPHY OF ANTERIOR THALAMIC NUCLEI AFFERENTS FROM THE POSTERIOR LIMBIC CORTEX AND THE CONTRALATERAL ANTEROVENTRAL NUCLEUS. J.M. Wyss, Th. van Groen* and C. Rodenburg* (SPON: S. Lane). Dept. of Cell Biology and Anatomy, Univ. of Alabama, Birmingham, AL. 35294 and Dept. of Pharmacology, Univ. of Edinburg, Scotland.

The afferent projections to the anterodorsal (AD), anteroventral (AV) and laterodorsal (LD) nuclei of the thalamus were studied by placing small iontophoretic deposits of Phaseolus vulgaris leucagglutinin (PHA-L) into the retrosplenial (Rg) and subicular cortices and the anterior thalamic nuclei of the rat. Injections into the postsubiculum labeled an ipsilateral terminal field in AD and ventral LD. This projection was topographically organized with more posterior areas of postsubiculum projecting to more lateral parts of LD and more dorsal regions of AD. In contrast, injections into the adjacent Rg labeled a topographically organized terminal field ipsilaterally in dorolateral LD and bilaterally in AV. PHA-L injections into the presubiculum labeled terminals ipsilaterally in dorsal LD, medial to the Rg projection, and bilaterally in dorsal AV. The projection from the parasubiculum terminated in AD. Injections of PHA-L into the anterior thalamic nuclei demonstrated that AV neurons projected to contralateral AV, but that neither AD nor LD had contralateral thalamic projections. These results emphasize the diversity of projections to the anterior thalamic nuclei, and suggest that each area likely has a different contribution to learning and memory.

373.3

THE CONNECTIONS OF THE CINGULATE CORTEX IN MICE: A DEGENERESCENCE AND HRP STUDY. M. Meunier, J. Villalobos and C. Destrade. (SPON: European Neuroscience Association). Lab. Psychophysiologie, UA CNRS 339, Univ. Bordeaux I, 33405 TALENCE FRANCE.

The afferent and efferent projections of the frontomedial, anterior and posterior parts of the cingulate cortex in BALB/c mice, were studied by analysing silver impregnation of the anterograde degeneration after ibotenic acid lesions in this area and by observing the retrograde axonal transport of WGA-HRP complex. The three cingulate regions have numerous common efferent projections particularly in the other cortical areas, striatum, the ventral thalamus and hypothalamus as well as in the mesencephalon. However, there are some differential connections. In particular, each of the 3 parts of the cingulate cortex shows reciprocal projections with one thalamic nucleus (the frontomedial part with the mediodorsal n., the anterior part with the anteromedial n. and the posterior part with the anteroventral n.). Amygdala appears to be connected with both the frontomedial and the posterior parts, whereas the subicular complex is selectively connected with the posterior cingulate cortex. These results show that the whole cingulate cortex is widely connected with non-limbic structures. In contrast, each part of the cingulate cortex is part of different limbic pathways.

373.4

MORPHOLOGICAL CHARACTERIZATION OF ENTORHINAL NEURONS IN THE RAT: AN INTRACELLULAR HRP STUDY. K. Lingenhöhl* and D.M. Finch (SPON. J. Lieb). Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Intracellular HRP injections were performed in entorhinal neurons. Stained neurons were found in layers III, IV and V. The dendritic trees of two layer III neurons were reconstructed. The primary dendrites of one cell, originating from a triangular soma, started ramifying close to the soma and extended into every layer. The dendrites of the second cell arose from a pyramidal shaped cell body with basal dendrites extending only into layer III. The apical dendrite branched close to the soma and extended with several branches into layer I. The axons of six layer III neurons were reconstructed in part. All of these axons showed a similar course. After crossing the deep layers the axons moved first medially into the angular bundle (AB) before projecting into the subiculum (S). Collaterals were found in the entorhinal cortex, the AB and the S. Most of the axons could be traced into the molecular layer of the S before fading. Only one axon was observed to cross the hippocampal fissure before fading. Three other neurons in or near layer IV showed the same axonal projection pattern. Two layer IV neurons were stained. Most of the dendrites bifurcated in layer IV and V. Only one apical dendrite extended into layer I, without branching out in layer III but with bifurcations in layer II and I. The axons gave off several collaterals in the entorhinal cortex and projected several thin branches ventro-laterally into the AB before fading. Three other neurons in or near layer IV showed the same axonal projection pattern. Two layer V neurons were stained. The apical dendrites showed a similar pattern as in the layer IV neurons, whereas the basal dendrites bifurcated to a lesser extent. Local axonal branches were present, but no major axonal branch was observed leaving the entorhinal cortex.

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373.5

TEMPORAL POLE PROJECTIONS TO THE MAGNOCELLULAR MEDIAL DORSAL NUCLEUS. E.C. Gower (SPON: J. Kucera). VA Medical Center, Boston, MA 02130.

Efferent projections from rostral temporal lobe cortices to the magnocellular medial dorsal nucleus (MDmc) were studied in the monkey (Macaca mulatta) with intracortical injections of horseradish peroxidase (HRP) and tritiated amino acids (TAA). TAA injections into pyriform allocortex or the transitional neocortical fields of the temporal pole (periallocortex and proisocortex) produced discrete zones of axonal termination in MDmc characterized by bursts of coarse label surrounding neuronal perikarya and their proximal dendrites. Although the same anterograde field was observed when HRP was injected into the transitional cortices, perikarya within the terminal clusters were not retrogradely labeled. Label was not transported to MDmc when injections were made into the superior or middle temporal gyri, and thus restricted to the isocortex. These findings indicate that a non-reciprocal corticofugal pathway to MDmc originates in the phylogenetically older districts of the temporal pole. This pathway constitutes an additional temporal route to MDmc parallelling those contributed by the amygdala and the hippocampal formation. The conduction of limbic sensory information directly from temporal neocortex to the medial thalamus may play a fundamental role in memory. (Supported by VA Medical Research funds.)

373.6

CORTICAL PROJECTIONS TO ORBITOFRONTAL LIMBIC CORTICES IN THE RHESUS MONKEY. H. Barbas. Depts. of Health Sci. and Anatomy, Boston Univ. and Sch. of Med., Boston, MA 02215.

Ipsilateral cortical projections to the least architectonically differentiated basal periallocortical, and to the slightly more differentiated proisocortical prefrontal areas were studied with the use of the retrograde transport of horseradish peroxidase (HRP). These areas, which exhibit an incipient laminar differentiation, received most of their projections from other limbic cortices. While most of the projections originated in nearby orbital and medial limbic prefrontal areas, HRP-labeled neurons were also found in temporal polar, perirhinal, prorhinal, entorhinal, agranular insular, and rostral cingulate limbic cortices, which lie at the origin of differentiation of the sensory and motor cortical systems. The basal limbic areas also received some projections from the more differentiated rostral auditory, visual, and somatosensory isocortices. The orbital periallocortex had fewer links with isocortices than the proisocortex.

The results indicate that orbitofrontal limbic cortices have widespread connections with areas which are found at the onset of differentiation of several sensory cortical systems, suggesting that they may have evolved at the same time.

(Supported by NIH grant NS24760).

373.7

CONVERGENCE OF AFFERENTS FROM MEDIAL PREFRONTAL CORTEX AND MAMILLARY BODY IN MEDIAL PONTINE NUCLEI OF THE RAT. G.V. Allen and D.A. Hopkins. Dept. of Anatomy, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

The pontine nuclei receive afferent fibers from limbic regions of the cerebral cortex and from the hypothalamus. In order to provide a better understanding of functional links between the limbic system and the cerebellum a light and electron microscopic study of limbic system connections with precerebellar relay nuclei has been undertaken.

Stereotaxic injections of 5% WGA-HRP were made into cortical, hypothalamic and brain stem sites. After three days, rats were perfused with a buffered aldehyde fixative. For light microscopy, frozen sections were incubated for reaction product in tetramethyl benzidine (TMB). For electron microscopy, vibratome sections were incubated in TMB followed by a second incubation in diaminobenzidine (Rye et al., '84). After injections into the medial pontine nuclei, retrograde labeling was present in the medial prefrontal cortex and the mamillary body. After injections into the medial prefrontal cortex or the mamillary body, dense anterograde labeling was found in the medial part of the pontine nuclei. The distributions of labeling from these two sources overlapped extensively in the pontine nuclei. Axon terminals in the medial pontine nuclei which originated from the medial prefrontal cortex and the mamillary body contained small round vesicles and formed asymmetric synapses with small diameter dendrites.

The present results demonstrate that afferents from the prefrontal cortex and the mamillary body with similar morphological characteristics and synaptic organization converge on neurons in the medial pontine nuclei which in turn project to vermal regions of the cerebellum. Thus, impulses from the limbic system may influence the activity of cerebellar regions which are known to be involved in certain autonomic functions.

Supported by MRC of Canada.

373.8

NEUROPHYSIOLOGICAL MEASURES OF HUMAN LIMBIC SYSTEM CONNECTIONS. C.L. Wilson, M. Isokawa-Akesson, R.C. Frysinger, S.U. Khan, T.L. Babb. Depts. of Neurology and Anatomy and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Limbic system connections have been analyzed intensively in subprimate species using both anatomical and electrophysiological methods, but since experimental anatomical methods cannot be used in human brain, functional studies must be used to assess connections. Buser and Bancaud (EEG J., 55:1-11, 1983) studied connections between hippocampus (HP) and amygdala (AM) using stimulation of depth electrodes implanted in these structures to monitor epileptic activity in medically intractable patients who were candidates for surgical seizure therapy. They reported mean onset latencies of 18-19 msec between HP and AM.

Studying a group of 31 patients with complex-partial epilepsy, we delivered single-pulse biphasic stimuli 100 μsec/phase in duration to each of five limbic electrodes in turn, while recording evoked field potentials from adjacent electrodes located in AM, anterior HP(aHP), middle HP(mHP), Entorhinal Cortex (EC), Presubiculum, and posterior Parahippocampal Gyrus. Latency and amplitude measures were made of averaged responses to 3-50 stimuli recorded from 4 to 9 sites. During AM stimulation, onset latencies to aHP were shorter (7.1 ms) than the previous study while to mHP they were longer (24.8 ms), suggesting their HP electrodes were targeted at a location between our aHP and mHP sites.

Comparison to other species must be on the basis of conduction velocities (CV), which have not previously been reported in human limbic sites, and therefore 25 CVs were calculated for the connection between each stimulation and recording site. CVs ranged from 0.88 m/s in the AM to EC pathway, to 3.4 m/sec in the perforant path connection from EC to mHP. This was followed closely by a 3.2 m/s CV in the longitudinal association connection from mHP to aHP. Our results are consistent with a trend toward slower rates of conduction higher on the phylogenetic scale, associated with a second trend of decreased fiber diameter and increased number of fibers in primates. NIH grant NS 02808.

373.9

COHERENCE OF EEG BETWEEN NEOCORTEX AND LIMBIC SYSTEMS. C.C. Turbes and G.T. Schneider*. Dept. of Anatomy, Creighton Univ. Sch. of Med., Omaha, NE 68178.

These studies are concerned with the interaction of spontaneous slow wave field potentials (EEG) and evoked potentials between nucleus accumbens, amygdala, septum and frontal, temporal and occipital cortex.

Twenty-four cats are used under nonanesthetic states utilizing telemetry and hardwire recording methods. Analog data is collected on FM tape and processed with a minicomputer. Coherence, partial coherence, phase spectral and cycle time analyses are used on the analog data.

These analyses show the degree of phase locking (coherence) between brain regions. Phase spectra and partial coherence processing show the directionality of neural signal flow between brain areas. Cycle time computations are used to show interaction in the time domain. Comparisons are made with pertinent anatomical data to determine the fit between the frequency and time domain plots and neural network functions. An example of a chemical altered state data is used to show how drugs alter brain region interaction.

373.10

SUBDIVISION AND NEURONAL TYPES IN THE INTERPEDUNCULAR NUCLEUS OF MAN. E.Braak and H.Braak. Dept. Anatomy, J.W. Goethe Univ. D-6000 Frankfurt 70, FRG

In series of sections cut at various thicknesses in the sagittal, frontal and horizontal plane through the human interpeduncular nucleus several subnuclei were delineated on account of lipofuscin pigmentoarchitectonic and cytoarchitectonic criteria. Previous investigations revealed that nerve cell types in the human adult can reliably be characterized by their lipofuscin pigment pattern.

The interpeduncular nucleus extends from the level of the caudal pole of the red nucleus to the level of the frontal pole of the locus coeruleus. It is about 8 mm long, 2 mm broad and 1 mm high. A medial subnucleus extends from the frontal to the caudal pole and contains 2 types of small non-pigmented neurons and 3 types of sparsely pigmented neurons. In the frontal half, additionally a scattered population of richly pigmented neurons occurs. Within the frontal lateral subnucleus small sparsely pigmented and non-pigmented neurons are intermingled with large non-pigmented nerve cells showing distinct Nissl bodies. At the frontal level of the pons, a small lateral cell group can be outlined harbouring small neurons with numerous faintly stained lipofuscin granules. Caudally, this subnucleus is replaced by a group of medium-sized neurons with numerous lipofuscin granules distributed between the elongated Nissl bodies.

Supported by the Deutsche Forschungsgemeinschaft.

373.11

STIMULATION OF LATERAL SEPTUM: IS IT ANXIOLYTIC? E. Yadin, E. Thomas, T.N. Holt*, and R.A. Hunt*. Department of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

Rats were trained on a water licking conflict procedure in which unpunished and signalled punished periods, each 2 min in duration, were alternated and cycled through twice. The punished period was signalled by tone and every 5th lick was accompanied by a 0.3-0.4 mA footshock. The number of licks was recorded throughout the 8 min session. After a stable baseline was achieved, rats were anesthetized and implanted with bipolar stimulating electrodes in the lateral nucleus of the septum or in the MFB.

After a week's recovery, animals were retested on the conflict test. The next phase consisted of conflict testing sessions during which continuous biphasic square wave stimulation (pulse duration 0.1 msec) was administered. The currents were adjusted individually so as not to produce convulsions and ranged between 75 and 500 μA.

Septal stimulation appeared to mimic the effects of anxiolytic agents. Thus during the first set of sessions at these currents septal stimulation produced increased responding in the punished periods and decreased responding in the unpunished periods whereas MFB stimulation did not. After several replications and raising of current levels in the MFB group, some of these animals displayed increased punished responding as well.

373.12

THE ELECTROPHYSIOLOGICAL RESPONSES OF MEDIAL CORTICAL NEURONS (AREAS 4,24,29) TO STIMULATION OF THE BASAL FOREBRAIN OF THE RAT. T.D. White*, A.M. Tan* and D.M. Finch (SPON: M.Nuwer). Brain Research Institute and Reed Neurological Research Center, University of California, Los Angeles, CA 90024.

Numerous anatomical studies have shown that the neocortex is a major target of the basal forebrain. However, the action of basal forebrain afferents on neocortex has not been described. This study presents the first in vivo intracellular characterization of synaptic responses of medial cortex to basal forebrain stimulation. Our sample of responsive neurons included 72 pyramidal cells and two fast-spiking, putatively nonpyramidal cells. The predominant synaptic response of cortical neurons was inhibition (55.4%). Reciprocal connections between recording and stimulating sites was indicated by a high incidence of antidromic action potentials (71.6%). Orthodromic potentials and antidromic spikes were generally observed in ipsilateral layer V neurons. The fast-spiking cells exhibited similar electrophysiological characteristics to previously described inhibitory interneurons, however, one produced an IPSP and both produced antidromic spikes following stimulation (diagonal band of Broca). Supported by NIH grant NS 23074.

373.13

INITIAL EXTRACELLULAR ELECTROPHYSIOLOGICAL CHARACTERIZATION OF CHOLINERGIC BASAL FOREBRAIN NEURONS. R. T. Matthews, Dept. of Anat., Texas A&M Univ., College Station, TX 77843.

Previous work has shown that a population of presumed cholinergic (Ch; AChE-positive) neurons of the medial septum/diagonal band (MS/DB) region of guinea pigs can be electrophysiologically distinguished from non-Ch neurons by intracellular recording in vitro (Griffith and Matthews, Neurosci. Lett. 71:169, 1986). Prominent features of Ch neurons include: 1) long duration action potentials (AP), 2) slow firing rate when depolarized by current pulses, 3) presence of an inward Cd^{+2}-sensitive Ca^{+2} conductance resulting in a shoulder on the repolarization phase of the AP and 4) presence of a late Ca^{+2}-dependent K^+ conductance resulting in a long duration post-spike afterhyperpolarization. Extracellular unit recordings from the same slice preparation yield extracellular representations of three of these four intracellular characteristics. Thus a subpopulation of MS/DB neurons (13 of 54) demonstrated: 1) long duration AP's (3.1 ± 0.27 ms versus 1.7 ± 0.13 ms for other neurons), 2) low maximum firing rate when driven by microiontophoresed glutamate (12-17 Hz versus up to 120 Hz for other neurons), 3) a multiphasic AP shape with significant shortening of the AP in a 0 Ca^{+2}/100 μM Cd^{+2} buffer (0.5 ± .07 ms decrease versus 0.1 ± .08 ms decrease for other neurons). It is concluded that at least some Ch neurons of the MS/DB can be distinguished from non-Ch neurons with extracellular recording techniques.

373.14

HETEROGENEITY OF BASAL FOREBRAIN CONNECTIONS IN THE RHESUS MONKEY. K.K. Hreib and D.L. Rosene. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

Our studies on the basal forebrain (BF) have revealed a complex array of connections that correspond to two major subdivisions: 1) magnocellular neurons comprising the medial septum (MS), vertical diagonal band (VDB), horizontal diagonal band (HDB) and nucleus basalis (NB); 2) parvicellular neurons comprising the lateral septum (LS), intermediate septum (IS), ventral pallidum (VP), ventral striatum (VS) and olfactory tubercle. BF afferents to the magnocellular medial dorsal thalamic nucleus originate mainly from cholinergic neurons in the MS, VDB and NB but also from non-cholinergic neurons in the VP. The major afferents to the NB, HDB, ventral VDB and ventral IS originate from distinct subsets of amygdalar nuclei but those to the MS-VDB, LS and dorsal IS originate from the hippocampal formation. The major afferents to VP originate from the nucleus accumbens, lateral basal (LB) and medial basal amygdala while those of the VS originate from the LB. Cortical afferents to NB and ventral VDB originate almost exclusively from the medial frontal cortex while entorhinal, perirhinal, inferotemporal and insular cortices project to the VS. These distinct patterns of connections suggest an equal diversity of function. (Supported by NS19416 and AG04321.)

373.15

CONFLICT BEHAVIOR IN RATS WITH LESIONS OF THE AMYGDALA, MAMMILLARY BODIES, LOCUS COERULEUS, AND DORSAL RAPHE AND THE EFFECTS OF ANXIOLYTIC DRUGS. H. Grishkat* and E. Thomas. Department of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

Animals were trained in a conflict test in which they drank for 2 min during unpunished segments which were alternated with 2 min punished segments. The punished periods, signaled by a tone, resulted in mild footshock (0.3-0.6 mA, 1 sec duration) for every fifth lick. The number of licks was recorded for all two minute segments throughout the 8 min session. Animals were trained to a stable baseline of suppression during the punished segments and were then subjected to bilateral electrolytic lesions of the amygdala, mammillary bodies, dorsal raphe, and locus coeruleus. One week after surgery animals were retested in the conflict paradigm for 5 sessions and then administered chlordiazepoxide (10 mg/kg) with two replications.

Approximately half the animals showed some release of suppression during the signaled, punished periods after lesioning. Administration of chlordiazepoxide produced a large release of punished behavior with no effect on unpunished responding. The results argue against these proposed anxiogenic areas as primary sites of action of the benzodiazepines.

373.16

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF SINGLE UNIT ACTIVITY IN THE NUCLEUS ACCUMBENS OF THE FREELY MOVING RAT. M.O. West and M. Wolske* (SPON: J. Perhach) Dept. of Psychology, Rutgers University, New Brunswick, NJ 08903.

Previous electrophysiological studies of extracellular activity in the nucleus accumbens have used anesthetized preparations. The purpose of the present study was to examine both single unit activity and field potentials in unanesthetized, freely moving rats. Long-Evans rats (300-350g) were chronically implanted with a detachable, miniature microdrive stereotaxically placed over the nucleus accumbens with reference to bregma (A-P 2.2mm , M-L 1.5mm, D-V 5.8-8mm). The synaptic input to the accumbens from the subiculum could be activated via an electrode implanted either in the ipsilateral fimbria-fornix or subiculum. Units (n = 82) were characterized in terms of waveform, spontaneous activity, synaptically activated response latency, and response to treadmill locomotion. The typical waveform was triphasic, with an initial negativity (200ms duration). Of the synaptically activated units, 58% were spontaneously active (range = 0.2 - 18.5 Hz, $\bar{x}$ = 3.3 Hz). At threshold stimulus intensity, 90% showed a short latency activation (5-11ms). Of these, 24% showed an additional long-latency activation (11ms). The remaining 10% responded only at long latencies, regardless of stimulus intensity. Of the units observed during treadmill locomotion, 91% showed increased firing rates ranging from 1-656% (compared with resting behavior), while 9% showed decreases ranging from 13 to 24%. Since the nucleus accumbens is a major target of ventral tegmental area neurons containing dopamine (a neurotransmitter linked with motor behavior), these studies are significant in that, in addition to eliminating any contaminating influences of anesthesia, they are performed during unrestrained movement such as locomotion, with which the accumbens has been implicated. Beyond this, neural activity patterns that are characterized in these studies can serve as substrates in pharmacological and behavioral studies (Wolske and West, Neurosci. Abstr., this volume). Supported by DA 04551, PHS RR 07058-21, NSF BNS-8708523.

374.1

DIURNAL RHYTHM OF β ADRENOCEPTORS IN THE CORTEX AND HYPOTHALAMUS OF C3H/HeN AND C57BL/6 MICE. E. Reuveny* and M. L. Dubocovich (SPON: C. A. Berry). Dept. Pharmacol., Northwestern Univ. Med. School, Chicago, IL 60611.

In this study we measured the diurnal variations in density and affinity of β adrenoceptors in cortical and hypothalamic membranes from C3H/HeN and C57BL/6 mice, using 3-[^{125}I]-Iodocyanopindolol (ICYP). Mice were housed in a 14:10 h L:D cycle and sacrificed at 3 h intervals over a 24 h period. The binding of ICYP to C3H/HeN mouse cortex membranes was of high affinity, reversible and saturable. ICYP (10-400 pM) binding defined with (±) propranolol (1 μM) was to a single site as determined by saturation analysis. In hypothalamic membranes from C3H/HeN mouse sacrificed at 0600 h and 1800 h the Kd (pM) values were 62 ± 10 (n=4) and 67 ± 12 (n=4), and B_{max} (fmol/mg protein) values were 161 ± 11 (n=8) and 121 ± 10 (n=7) ($p<0.05$), respectively. In cortical membranes from C3H/HeN mouse as in hypothalamic membranes, the Kd values did not change over the 24 h period [Kd=35 ± 6 (n=4) and B_{max}=182 ± 6 (n=4) at 1200 h], however the β receptor density was significantly higher during the dark period [B_{max}=226 ± 17 (n=4), $p<0.05$]. In contrast, in mouse hypothalamic membranes from C57BL/6 no diurnal variations in Kd or B_{max} were observed [Kd=59 ± 6 n=4; B_{max}= 119 ± 4 (n=4) at 1800 h]. The diurnal variations in the density of β adrenoceptors observed in the hypothalamus of C3H/HeN but not in C57BL/6 mice may be attributed to existing genetic differences. Supported by DK 38607.

374.2

DOES AGE ALTER THE NUMBER OF BETA RECEPTORS IN LYMPHOCYTE MEMBRANES? D.W. Gietzen, T. Goodman*, J.R. Magliozzi, R. Maddock*, and P.G. Weiler*, Depts of Psychiatry & Community Health, School of Medicine, University of Calif., Davis, CA 95616.

Evidence regarding the effects of age on beta receptor binding in lymphocytes has been controversial. In order to address this issue, we used both ^{125}[I]-cyanopindolol (ICYP) and ^{3}[H]-dihydroalprenalol (DHA) as ligands in separate studies carried out over a 2 year period. The ages of subjects ranged from 18-90 years, with a bimodal distribution, having subjects predominantly in young and old groups. Mean ages (± SE; N = number of subjects) were: DHA old gp: 63.8±3.9 (N=35), young gp: 23.4±0.9 (N=21); ICYP old gp: 70.3±1.9 (N=24), young gp: 29.6±2.1 (N=20). Potential subjects were excluded for physical or psychiatric illness. Lysates of lymphocyte membranes were prepared from freshly drawn venous blood according to a modification of the method of Brodde et al. (Life Sci 29:2189, 1981), and used in binding assays with either DHA or ICYP. Scatchard analysis gave estimates of binding maxima (Bmax) and antagonist affinity (Kd). In both studies, increases in Bmax, with no change in Kd, were found in the older groups. Values for Bmax were: DHA old gp: 64.8±3.9, young gp: 48.6±4.0; ICYP old gp: 81.4±11.7, young gp: 47.2±5.6. The increases in Bmax were highly significant (for DHA, F:1,35 = 8.79, $p<0.01$; for ICYP, F: 1,41 = 19.06, $p<0.001$). Thus, in these preparations, the binding of beta antagonists to lysates of lymphocytes was increased with age. Supported by N.I.Aging Grant # K07AG0023003 and McNeil Pharmaceutical, Inc.

374.3

THE ONTOGENY OF α_1- AND β_1-ADRENERGIC RECEPTORS IN MOUSE FOREBRAIN: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. C.D. Searles*, H.S. Singer and J.T. Coyle. Depts. of Neurol., Neurosci. and Ped., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Norepinephrine appears to have an important role in the development of the mammalian brain. The transmitter activates at least 4 receptor classes in mammalian brain, designated β_1, β_2, α_1 and α_2, but neuronal functions are most closely related to α_1 and β_1 receptors. This study is a quantitative autoradiographic analysis comparing the appearance and distribution of α_1 and β_1 receptors in mouse forebrain using the radioiodinated ligands HEAT ([^{125}I]hydroxyiodophenylethylaminomethyl tetralone) and ICYP ([^{125}I]iodocyanopindolol) to determine the possible differential expression of these two receptors.

Specific binding of HEAT first appears in mouse forebrain on E16, two days later than ICYP binding. Late in gestation (between E16 and E18), α_1 receptors are restricted to the basal forebrain, in contrast to β_1 receptors, which are present in striatum, cortical plate, and basal forebrain. Early in the postnatal period (P6), moderate densities of HEAT binding sites are present in the striatum, neocortex (diffuse in anterior-medial regions but limited to inner regions more laterally), and thalamus (ventral nuclei). By P15, the adult distributional pattern of α_1 receptors has developed, and on P20 the highest densities of receptor binding have been attained: about 40 fmol/mg tissue are present in frontal and anterior cingulate cortex, layer VI of lateral cortex, basal forebrain and nuclei of the dorsal thalamus. Specific binding is low in the striatum and hippocampal formation. For β_1 receptors, the adult pattern of receptor sites is present at P10, with highest concentrations of receptors found in the cortex, striatum and hippocampus.

This study demonstrates differences in the development, distribution and density of α_1 and β_1 receptors in mouse forebrain. These findings suggest different roles for these two receptors in mediation of adrenergic functions in the developing brain.

374.4

COMPARISION OF ^{3}H-DIHYDROALPRENOLOL AND ^{3}H-CGP12177 FOR MEASURING CNS ß-ADRENERGIC RECEPTORS. M. Riva and Ian Creese. Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102.

^{3}H-Dihydroalprenolol (^{3}H-DHA) is one of the most frequently used radioligands for measuring CNS ß adrenergic receptors . Usually the non-specific binding for ^{3}H-DHA is measured using a very high concentration (10-20μM) of a ß adrenergic receptor antagonist, such as propranolol or alprenolol. However these compounds, at this high concentration, have been demonstrated to have a serotonergic component to their binding. In our studies the competition curve of (-)alprenolol for ^{3}H-DHA binding is better fitted to a two site model than a one site model. The Ki of alprenolol for the first subpopulation of sites labeled by ^{3}H-DHA is 1-2 nM while the Ki for the second portion is 0.5-1μM. The lower affinity site for alprenolol is in accordance with its affinity for ^{3}H-5HT binding sites measured by other investigators. These results imply that, even at low concentrations, ^{3}H-DHA must also label a serotonin receptor. We are attempting to determine which serotonin receptor subtype is involved. Neither 8-OHDPAT ($5HT_{1A}$) nor mesulergine ($5HT_{1C}$) are able to displace ^{3}H-DHA binding at low concentrations, while RU24969, a specific $5HT_{1B}$ ligand displaces 15% of ^{3}H-DHA binding with a Ki = 0.5-1 nM that is in accordance with its affinity for the $5HT_{1B}$ receptors. Experiments are in progress in order to determine if the increase of ^{3}H-DHA binding following serotonergic depletion or lesion of 5HT neurons is due to a change in $5HT_{1B}$ receptors as opposed to an increase in ß receptors. We have been using another radioligand, ^{3}H-CGP12177,to measure ß receptors. This radioligand has some advantages over ^{3}H-DHA. Being hydrophilic, it has a very low non-specific binding and competition curves of alprenolol for ^{3}H-CGP12177 are monophasic indicating that it labels only ß receptors. This could be helpful in discriminating the modifications occurring in ß receptors following treatment with antidepressant drugs.
Supported by NIDA DA04612 and NIMH MH00316.

374.5

Cytoplasmic Loop of β-Adrenergic Receptors: Synaptic and Intracellular Localizations in the Neocortex, Striatum and Brainstem of Rat Brains Using a Monoclonal Antibody. C. Aoki[1], B.A. Zemcik*[2], C.D. Strader[2] & V.M. Pickel[1]. [1]Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, N.Y. 10021 and [2]Dept. of Biochem. & Mol. Biol., Merck Sharp & Dohme Research Lab., Rahway, New Jersey, 07065.

A monoclonal antibody, βAR(226-239)3-1, against amino acids #226 - 239 of hamster lung β-adrenergic receptor (βAR) (β_2-subtype) has been biochemically characterized to be monospecific to βAR of smooth muscle DDT-1 cells and hamster lung membrane (Zemcik & Strader, Biochem. J., in press, 1988). Further, by analogy with other G protein-linked proteins, its antigenic site is predicted to reside within a cytoplasmic loop between the fifth and sixth transmembrane helices of the protein. We used the ABC method (Hsu et al., J. Histochem. Cytochem. 29, 577, 1981) to ultrastructurally localize the antigenic sites for this antibody within the following areas: the neocortex which exhibits high densities of both noradrergic fibers and βAR; striatum, which exhibits a low density of fibers but a high density of βAR; and brainstem areas which contain high densities of noradrenergic fibers, cell bodies and βAR. In all areas examined, the cellular and subcellular distribution of immunoreactivity was similar. Labeling occurred in discrete cytoplasmic patches within neuronal perikarya and proximal dendrites, particularly in association with rough endoplasmic reticulum, dense-core vesicles and cytoplasmic surfaces of plasmalemma. At select axodendritic junctions, labeling also was detected at presynaptic membrane specializations, but were more numerous along thick and thin postsynaptic densities. The results provide information relevant to the synthesis of βAR and support previous pharmacological evidences for the existence of presynaptic βA-autoreceptors and noradrenergic modulation of excitatory and inhibitory synaptic transmission in these areas. (Supported by grants NIH NS07782 to CA; NIMH MH40342 & MH00078, NIH HL18974 and NSF BNS8023914 to VMP).

374.6

BETA-ADRENOCEPTOR DENSITY IN HARDERIAN GLANDS OF HAMSTERS SHOWS PRONOUNCED SEXUAL DIFFERENCES. B.Pangerl *, A.Pangerl *, D.J.Jones +, R.J.Reiter. Dept. Cell. Struct. Biol. & Anesthesiology +, Univ. TX Health Sci. Ctr., 7703 Floyd Curl Drive, San Antonio, TX 78284-7762

Harderian glands, large tubulo alveolar glands in the orbital cavity, are sympathetically innervated by the superior cervical ganglion and present in mammals having nictitating membranes. The beta-adrenoceptor density (Bmax) and the dissociation constant (Kd) of Harderian glands in adult male and female Syrian hamsters were estimated using a Scatchard saturation assay with 7 concentrations (30 pM-1200 pM) of the radioligand (-)[125I] Iodopindolol (I-PIN). Specific binding was saturable and varied between 60-85 % of total binding. Tissues were taken at 15:00 h from hamsters in LD 14:10 (lights on 07:00 h) and immediately frozen on dry ice. Bmax (fmol/mg protein ± SEM) was 16X higher in females vs. males (292 ± 45 vs. 18 ± 3, $p<0.001$) and the Kd (nM ± SEM) was 4X higher in females vs. males (1.08 ± 0.18 vs. 0.26 ± 0.05, $p<0.005$). This finding is the first report of beta adrenoceptors in Harderian glands and adds to the data on hydroxyindole-O-methyltransferase, melatonin and porphyrin contents which also demonstrate higher levels in females.

B.P. was supported by the Feodor-Lynen-Program of the Humboldt Society, work was supported by NSF grant DCB 8711241 and NIH grant 14546.

374.7

MPP+ INHIBITS LIGAND BINDING AT DOPAMINE, α_2-ADRENERGIC AND 5-HT_2 BINDING SITES IN HUMAN BRAIN. A.C. Andorn and J. Pickett*. F.D.R. V.A. Hospital, Montrose, N.Y. 10548 and Department of Psychiatry, Case Western Reserve University, Cleveland, Ohio.

MPP^+, an oxidative product of MPTP, is a selective dopaminergic neurotoxin. MPP^+ interactions with the dopamine reuptake system have been well documented. The effect of MPP^+ on dopamine, α_2-adrenergic and serotonin binding sites in striatum and cortex had not been previously reported. Using standard radioligand filtration binding assays, performing dose-response studies of a minimum of 22 points each, replicated, and using LIGAND to resolve the data, we observed that MPP^+ inhibited ligand binding at [^{3}H]spiroperidol ([^{3}HSP]) binding sites in postmortem human cortex (5-HT_2), putamen (dopamine$_2$) and at cortical [^{3}H]UK14,304 (α_2-adrenergic) binding sites. Shown below are the K_A for MPP^+ (the K_i are given in parentheses) at each of the radioligand affinity states detected.

	K_A (+/- SEM) (M^{-1})	
Binding Site	High Affinity	Low Affinity
[^{3}H]SP Cortex	$1.8\pm0.5 \times 10^5$ (5.4×10^{-6}M)	$6.6\pm1.3 \times 10^5$ (1.5×10^{-6}M)
[^{3}H]SP Putamen	$6.4\pm5.9 \times 10^5$ (1.6×10^{-6}M)	$9.3\pm20 \times 10^3$ (1.1×10^{-3}M)
[^{3}H]UK14,304	$2.9\pm1.6 \times 10^6$ (3.4×10^{-7}M)	$4.8\pm1.2 \times 10^4$ (2.1×10^{-5}M)

These findings suggest that MPP^+ has potentially complex interactions with neurotransmitter systems.

374.8

ASPARTAME TREATMENT DOES NOT AFFECT BRAIN AMINES AND RELATED RECEPTOR KINETICS IN RATS. M.A. Reilly*, E.A. Debler, A. Fleischer* and A. Lajtha. Ctr. for Neurochem., N.S. Kline Inst. Ward's Island, NY, NY 10035.

The use of the dipeptide sweetener aspartame (APM, L-aspartyl-L-phenylalanine methyl ester) represents an additional dietary source of phenylalanine (Phe), although plasma levels of this amino acid are not usually elevated for a long period of time after APM consumption. Since Phe and its metabolite tyrosine (Tyr) may influence some CNS neurotransmitter systems, it was of interest to investigate the effects of subchronic APM consumption on brain aminergic activity. Male Sprague-Dawley rats were given 50 or 500 mg/kg APM per day in drinking water for 30 days. Cerebral cortex and striatum were excised for determination of amine levels and for study of binding kinetics at several receptors: adrenergic α_1 (prazosin) and α_2 (clonidine), dopaminergic D_1 (SCH 23390) and D_2 (spiperone), and serotonergic $5HT_2$ (ketanserin). K_d and B_{max} values were estimated from 6 parallel determinations (2 animals pooled per determination), each consisting of 6 ligand concentrations. We found no significant changes in binding parameters for any of the receptors studied. In addition, no significant differences were found in brain dopamine, serotonin, and norepinephrine levels in our present experimental design. Our findings of no change in binding affinity and density lead to the conclusion that, in rats, APM treatment is unlikely to affect catecholaminergic or serotonergic activity.

374.9

MEASUREMENT OF EXTRACELLULAR cAMP IN BRAIN BY MICRODIALYSIS: A METHOD TO STUDY BRAIN RECEPTOR FUNCTION IN VIVO. E.A. Stone and M. Egawa*, NYU Med. Ctr., NY, NY 10016

We have shown previously that cAMP in the extracellular fluid of the brain can be detected using an implanted microdialysis probe (Trans. Am. Soc. Neurochem. 19:159, 1988; Faseb J. 2:A1800, 1988). In the present studies we have further developed this technique into a method for studying the function of noradrenergic and other cAMP-linked receptors in the intact brain. Urethane-anesthetized rats were implanted in the frontal cortex with microdialysis probes which were perfused with various agents for various periods. cAMP in the perfusate (dialysate) was assayed by RIA (sens. 2 fmol). In agreement with previous studies in brain slices, norepinephrine (NE), isoproterenol (ISO) and adenosine (10^{-6}-10^{-3} M) were found to induce dose-dependent increases in in vivo cAMP levels. The response to NE was blocked 80% by the beta antagonist, timolol. Infusion of the alpha receptor agonist, 6-fluoronorepinephrine, led to a potentiation of the responses to ISO and adenosine. Prolonged infusion of ISO caused a progressive desensitization of the cAMP response and the latter effect was partially reduced by prior treatment with corticosterone. The results are in accordance with previous in vitro studies and indicate that the microdialysis-cAMP method can be used to study the function of noradrenergic and other receptors in the brain in vivo. (Supported by MH22768 and MH08616).

374.10

PROGESTERONE MODULATION OF α AND β ADRENERGIC RECEPTOR INTERACTIONS IN HYPOTHALAMIC SLICES. N. Petitti and A.M. Etgen. Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461.

Norepinephrine (NE)-stimulated cyclic AMP (cAMP) accumulation in hypothalamic and preoptic area slices was monitored to examine the effects of progesterone (P) on brain adrenergic receptor coupling to adenylate cyclase. P treatment decreased NE-induced slice cAMP accumulation. This effect was dependent on prior estrogen exposure and was independent of increases in phosphodiesterase activity or decreases in adenylate cyclase activity. In all slices cAMP levels were elevated by isoproterenol, a β receptor agonist, but not by α_1 (phenylephrine) or α_2 (clonidine) agonists. However, in slices from estrogen plus P-treated rats, clonidine potentiated the effect of isoproterenol on cAMP formation whereas phenylephrine did not. In contrast, phenylephrine but not clonidine enhanced isoproterenol-induced cAMP accumulation in slices from rats receiving only estrogen. In P-exposed slices, NE-stimulated cAMP accumulation was completely antagonized only by a combination of both β (propranolol) and α_2 (yohimbine) antagonists. The data suggest that P treatment of estrogen-primed rats (1) depresses NE-stimulated cAMP accumulation in hypothalamic and preoptic area slices, (2) decreases or eliminates α_1 receptor facilitation of cAMP synthesis, and (3) promotes an α_2 receptor augmentation of β receptor stimulation of adenylate cyclase.

374.11

ADRENERGIC MODULATION OF THE K^+ CONTRACTURES IN TONIC MUSCLE FIBERS OF THE FROG. M. Huerta, J. Muñiz* & X. Trujillo*+. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima. Apdo. Postal 199, 28000 Colima, Col., México.

Tension was isometrically recorded from tonic bundles of cruralis muscle of Rana pipiens. Normal solution was (mM): NaCl 117.5, KCl 2.5, $CaCl_2$ 1.8. pH was adjusted to 7.4 with Imidazole chloride. Experiments were done at room temperature (20-22°C). Epinephrine and Isoproterenol were added from stock solutions. The solutions contained d-tubocurarine (50 uM). In Ca^{++}-free solution $CaCl_2$ was replaced by $NiCl_2$ (0.5 mM). The bundles generated tension while soaked in high K^+ solution and relaxed when they were returned to the normal solution. When tonic bundle was incubated for 30 minutes in Epinephrine (20-40 uM) it enhanced K^+ contractures by approximately 50% (n=4). Similar results were obtained with isoproterenol (1 uM) (n=5). After 10 minutes in Ca^{++}-free solution the peak tension was greatly reduced and sustained component was abolished, but when Epinephrine (20 uM) was added in Ca^{++}-free solution only the peak tension was enhanced by approximately 600% (n=6). These actions were reversible. These results demonstrated the existence of adrenergic receptors in tonic muscle related to the regulation of developed tension.

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+ Conacyt Felloship.

374.12

ADRENERGIC RECEPTOR FUNCTION IN DEPRESSION. E. S. Werstiuk and M. Steiner. Department of Neurosciences and Psychiatry, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Functional alterations in adrenergic receptors have been implicated in depression, and treatment with antidepressants is proposed to lead to modulation of receptor sensitivity. The present study was undertaken to assess the effect of ECT on leukocyte and platelet adrenergic receptor functions in depressed patients. Subjects were 12 acutely depressed patients treated with ECT. 12 healthy subjects served as controls. We measured adrenergic receptor binding parameters (B max and Kd) in intact platelets (3H-Yohimbine) and in intact leukocytes (^{125}I-Cyanopindolol), and receptor function, β-receptor stimulated cAMP levels in leukocytes, α_2-receptor-mediated inhibition in platelets, pre-treatment and 14 days after the last ECT. Pre-ECT receptor binding parameters were not significantly different from those of controls, and treatment had no significant effect. Receptor mediated cAMP levels increased in both platelets, and leukocytes, following ECT, suggesting an enhanced functional response. Whether these changes are specific to ECT, or result from alterations in circulating catecholamines will require further clarification.

Supported by the Ontario Mental Health Foundation.

375.1

EFFECTS OF CHRONIC TREATMENT WITH SELECTIVE AGONISTS ON THE SUBTYPES OF DOPAMINE RECEPTORS. **Swaminathan Subramaniam, Irwin Lucki and Paul McGonigle. Dept. of Pharmacology, Univ. of Pennsylvania, Phila. PA 19008.**

The effects of chronic administration of selective agonists on D-1 and D-2 receptor density, affinity and function were measured in rats. Animals received 21 daily injections (i.p.) of the D-1 selective agonist SKF-38393, the D-2 selective agonist quinpirole, SKF-38393 plus quinpirole or vehicle. Serial 20 μm sections that included the caudate putamen (CPu) and nucleus accumbens (NAc) or the substantia nigra (SN) were cut and alternately labeled with ^{3}H-SCH-23390 and ^{3}H-spiroperidol. Treatment with quinpirole or quinpirole plus SKF-38393 decreased the density of D-2 receptors in the CPu (15%), NAc (15%) and SN (43%), whereas SKF-38393 had no effect on these sites. In contrast, treatment with SKF-38393 increased the density of D-1 receptors in the CPu (26%), NAc (22%) and SN (29%). Treatment with quinpirole alone had no effect on D-1 receptors, however, quinpirole attenuated the effect of SKF-38393 on these sites in the CPu and NAc. To ascertain the effects of chronic agonist treatment on functions mediated by each receptor subtype, quinpirole-induced depression of rectal temperature and SKF-38393-induced oral dyskinesias were measured before and after chronic administration of agonists. Treatment with quinpirole or quinpirole plus SKF-38393 resulted in a desensitization of the D-2-mediated depression of rectal temperature which was consistent with results of the biochemical measurements. None of the treatments produced desensitization of the D-1-mediated oral dyskinesias which is also consistent with the biochemical results. (Supported by USPHS grant GM 34781)

375.2

COMPARISON OF BEHAVIORAL AND BIOCHEMICAL CONSEQUENCES OF TWO DISTINCT MODELS OF CENTRAL DOPAMINERGIC DENERVATION SUPERSENSITIVITY B. E. Mileson and R. B. Mailman, Univ. of North Carolina Curriculum in Toxicology and Biological Sciences Research Center, Chapel Hill, NC 27599.

Behavioral and biochemical endpoints of two different models of dopaminergic denervation supersensitivity were compared in order to examine possible mechanisms of supersensitivity. Denervation of central dopamine neurons resulting in severe dopamine depletion was effected in rats by intracisternal (IC) administration of 6-hydroxydopamine (6-OHDA) (200 μg) or bilateral infusion of 6-OHDA into the substantia nigra (8 μg /side). Low-dose agonist challenge of recovered rats lesioned in the nigra elicited a number of behaviors not seen in sham lesioned control rats, including self-biting, intense grooming and ardent gnawing of the cage floor. IC-lesioned rats responded to agonist challenge by explosive locomotor activity and rearing. Receptor binding studies performed on striatal membranes of both lesioned and control rats revealed no change in the maximum number of binding sites (B_{max}) or the dissociation constant (K_d) of either D_1 or D_2 receptors for either lesioned group compared to control. Biochemical function of D_1 receptors was evaluated by measurement of dopamine-sensitive adenylate cyclase in striatal membrane preparations. Differences in dopamine stimulated adenylate cyclase activity due to lesion were slight, indicating additional mechanisms are involved in mediating behavioral supersensitivity. Supported by PHS Grants ES01104 and MH40537.

375.3

TEMPORAL CHANGES IN STRIATAL D_1 DOPAMINE RECEPTOR DISTRIBUTION AFTER DOPAMINE DENERVATION. T. DeLorenzo* & M.A. Ariano. University of Vermont College of Medicine Burlington, VT 05405.

A comparison of the morphochemical distribution of striatal D_1 dopamine binding sites, 3 days to 20 weeks following unilateral infusion of 6-OHDA into the substantia nigra, has been performed. Autoradiographic localization of the selective D_1 antagonist ligands ^{3}H-SCH 23390 or ^{125}I-SCH 23982 was assessed in relation to cyclic AMP immunochemically characterized striatal neurons (Br. Res. 443:204,1988). The extent of dopamine depletion in the striatum was determined biochemically or morphologically. All control striatae, contralateral to the 6-OHDA infusion, showed a specific clustered association of D_1 binding with cyclic AMP reactive neurons. In contrast, this morphochemical relationship was lost by 7 days post lesion on the denervated side, and remained uncoupled up to 4 weeks. At 5 weeks, reassociation of D_1 binding sites with cyclic AMP containing neurons occurred in the medial margin of the denervated striatum. This event became more pronounced with time (up to 20 weeks). Biochemical analyses of D_1 binding demonstrated gradual decrements in specific binding up to 4 weeks post-lesion, with a slight recovery after 5 weeks, in good agreement with the anatomical data. Our findings suggest a temporal alteration in D_1 receptor coupling to cyclic AMP reactive neurons following long term denervation of rat striatum.

375.4

CCK ANTAGONIST LORGLUMIDE BLOCKS ACUTE AND CHRONIC HALOPERIDOL-INDUCED EFFECTS ON DA NEURONS L.H. JIANG* AND R.Y. WANG. (SPON: Irwin Fand) Dept. of Psychiat. & Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794-8790

The present study examined the ability of lorglumide (LORG) to block acute and chronic HAL-induced effects on midbrain DA cells. LORG is a more potent and selective CCK antagonist than proglumide (PROG) in pancreas. Male Sprague-Dawley rats were treated with HAL for 3 weeks. Standard extracellular single unit recording techniques were used to determine the number of spontaneously active DA cells in anatomically defined A9 and A10 regions. Similar to the results obtained with PROG, i.v. LORG reversed the CHAL-induced reduction of DA cells/track in both A9 and A10. Moreover, microinjection of LORG (4.8 ng in 1 μl), but not naloxone, directly into the medial nucleus accumbens (mNAc), an area containing a high density of CCK/DA nerve terminals and receptors, dose-dependently reversed CHAL-induced effect. LORG injected into the lateral NAc or the caudate-putamen was without effect. In addition, i.v or microinjection of LORG into the mNAc also reversed acute HAL-induced firing rate increase of both A10 and A9 DA cells. These results strongly suggest that CCK receptors in the mNAc form an important link for maintaining HAL-induced effect on midbrain DA neurons and CCK is involved in the therapeutic action of antipsychotic drugs. (Supported by USPHS Grants MH-41440, MH-41696 and MH-00378 to R.Y.W.).

375.5

ENHANCED RESPONSES OF NUCLEUS ACCUMBENS (NAc) NEURONS TO DOPAMINE (DA) D_1 AND D_2 RECEPTOR AGONISTS FOLLOWING 6 MONTHS TREATMENT WITH ANTIPSYCHOTIC DRUGS (APDs). Xiu-Ti Hu and Rex Y. Wang, Dept. of Psychiatry & Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794-8790.

The present study using double blind design examined the effect of chronic APD treatment on the response of NAc cells to selective D_1 agonist (+)SKF-38393 and D_2 agonist quinpirole (LY). Male Sprague-Dawley rats were treated with either haloperidol (HAL, 1 mg/ kg/day), clozapine (CLOZ, 25 mg/kg/day) or water via their drinking bottles for 6 mo. The techniques of single-unit recording and microiontophoresis were used. At low ejecting currents (1-5 nA), both SKF and LY facilitated the excitatory effects of glutamate, whereas at higher ejecting currents (>10 nA) they inhibited the NAc activity. The inhibitory response of NAc cells to SKF and LY was significantly increased in both HAL and CLOZ-treated rats (with or without drug withdrawal period) compared to controls. Dose-response curves for both SKF and LY were shifted to the left in APD-treated groups. Moreover, a synergistic action of SKF and LY was observed for most cells tested in APD-treated rats but not in control rats. There was no significant difference in the effects produced by chronic HAL and chronic CLOZ on NAc cells, suggesting that the NAc may be importent in mediating the therapeutic action of APDs. (Supported by USPHS Grants MH-41440, MH-41696 and MH-00378 to R.Y.W.)

375.6

THE SENSITIVITY OF RAT CAUDATE-PUTAMEN (CPu) NEURONS TO D_1 AND D_2 RECEPTOR AGONISTS ARE NOT CHANGED BY LONG TERM TREATMENT WITH ANTIPSYCHOTIC DRUGS (APDs). R.Y. Wang, L.H. Jiang* and R.J. Kasser. Dept. of Psychiat. and Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794.

It is thought that tardive dyskinesia (TD) might be the result of enhanced dopamine (DA) receptor sensitivity following long-term APD treatment. The present study was designed to test this hypothesis. Male Sprague-Dawley rats were treated with either haloperidol (HAL, 1 mg/kg/day), clozapine (CLOZ, 25 mg/kg/day) or water via their drinking bottles for 6-10 months. Using a double-blind design, we found that there was no significant difference in the sensitivity of CPu neurons to iontophoresed SKF-38393 (a D_1 receptor agonist) or quinpirole (a D_2 receptor agonist) in control, HAL and CLOZ groups, although many of these APD-treated rats showed spontaneous oral movements. These results are consistent with the observations that DA-agonists induced changes in striatal cAMP and inositol phosphate were not different in control and APD-treated groups. They contrast markedly with the results from studies showing that compared to controls, there was a significant increase of D_2 receptor binding sites in the CPu following chronic APD treatment. In conclusion, our results do not support the view that TD is the result of DA receptor supersensitivity. (Supported by USPHS Grants MH-41440, MH-41696, MH-00378 to R.Y.W. and MH-43893 to R.J.K.)

375.7

EFFECTS OF CHRONIC ANTIPSYCHOTIC DRUG (APD) TREATMENT ON STRIATAL cAMP CONTENT IN RESPONSE TO D_1 AND D_2 RECEPTOR AGONISTS. C.R. Ashby, Jr., J.E. Rubinstein*, R.J. Hitzemann and R.Y. Wang. Dept. of Psychiat. and Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794-8790

The present study compared the effect of chronic APD treatment on striatal cAMP content in response to dopamine (DA) D_1 and D_2 receptor agonists (+)SKF-38393 (SKF) and quinpirole (LY). In addition, the effects of forskolin and LY plus SKF were examined. Male Sprague-Dawley rats were treated with haloperidol (HAL, 1 mg/kg/day), clozapine (CLOZ, 25 mg/kg/day) or water via their drinking bottles for 12 months. The neostriatal slices were incubated in oxygenated buffer with SKF (10^{-6}-10^{-4} M), LY (10^{-7}-10^{-5} M), forskolin (10^{-5} M) or LY+SKF (10^{-6} and 10^{-5} M) and cAMP content was measured using a RIA method. SKF increased and LY decreased cAMP content. There were no differences among groups in cAMP content in response to SKF, LY or forskolin. These results show that 12 mo. APD treatment failed to alter D_1 or D_2 receptor sensitivity. Additionally, the forskolin results indicate that the activity of adenylate cyclase was unchanged. However, in HAL treated rats, LY+SKF significantly increased striatal cAMP content compared to the water group, suggesting that the interaction between DA receptor subtypes may be differentially affected by chronic APD treatment. (Supported by USPHS Grants MH-41440 and MH-00378 to R.Y.W. and VA Res. Admin. to R.J.H.).

375.8

EFFECTS OF CHRONIC ANTIPSYCHOTIC ADMINISTRATION ON THE FORMATION OF INOSITOL PHOSPHATES IN RAT STRIATUM. J.E. Rubinstein*, R.J. Hitzemann, C.R. Ashby, Jr. and R.Y. Wang (SPON: J.G. May, III). Dept. Psychiatry SUNY: Stony Brook, NY 11794

Previous reports have suggested that muscarinic agents stimulate and D2 dopamine agonists inhibit the hydrolysis of polyphosphoinositides in rat striatum. We sought to replicate these findings and investigate whether long-term treatment with haloperidol (HDL) or the "atypical" antipsychotic clozapine (CLZ) modified these effects.

Male Sprague-Dawley rats were treated for 12 months with HDL, 1 mg/kg/day, or CLZ 25 mg/kg/day. After a 60 min preincubation in Krebs-Ringer-Bicarbonate buffer (KRB), striatal slices were labeled for 2 hr with 0.7 μm [^{3}H]-inositol in a final volume of 1 ml/100 mg tissue. 50 μl of labeled slices were added to KRB containing 10 mM Li^+ and test drugs (final volume = 0.5 ml). After 30 min, the incubation was terminated by the addition of 1.5 ml $CHCl_3$/MeOH/HCl (1:2:.01). All incubations were carried out at 37°C under an atmosphere of 95% O_2/5% CO_2. Inositol phosphates were separated by ion exchange chromatography.

Carbachol (0.5 mM) increased the accumulation of IP by 240% in both treatment groups as well as age-matched controls. IP2 and IP3 were increased 28-44% and 8-30%, respectively. Quinpirole, a selective D2 agonist, had no effect on IP or IP2 levels at concentrations of 0.1-10 μm. Paradoxically, IP3 was increased - minimally in controls, 15% in the CLZ group - however, this effect was not dose-dependent and did not reach statistical significance. This result conflicts with the report by Pizzi et al. (Soc. Neurosci. Abs., Vol. 13, #23.13, 1987) and may represent an effect of aging. The results are discussed in relation to parallel presentations of studies of DA receptor density, neuronal firing rate and adenylate cyclase activity in these chronically treated rats.

375.9

CYCLO(LEU-GLY) (CLG) PREVENTS NIGROSTRIATAL DOPAMINE (DA) SUPERSENSITIVITY (SS) IN MICE. JZ Fields[1], JM Lee*[2], JH Gordon[1] & RF Ritzmann[2], Res Svce Hines[1] & Brentwood[2] VA Hospitals, Hines IL 60141 & Olive View/UCLA Med Ctr, Los Angeles CA 91342

Pro-leu-gly-NH_2 (MIF-1), the C-terminal fragment of oxytocin, and its structural analog, CLG have been shown to alter, after in vivo administration, both DA mediated behaviors and D2 DA receptor binding. In our own studies, CLG coadministration to mice prevented the DA SS in the mesolimbic DA tract (apomorphine (APO) induced locomotion) induced by chronic haloperidol (HAL). In this study we evaluated the effects of CLG on nigrostriatal DA SS. Vehicle (I) or chronic HAL (II)(1.0 mg/kg, ip X 21 days) were given with or without various doses of CLG (sc): [a] 0 mg/kg/day, [b] 1 mg/kg every third day (30 min prior to HAL), [c] 1 mg/kg every day, or [d] 8 mg/kg every third day. Average CLG doses (mg/kg/day) were thus [a] 0, [b] 0.33, [c] 1.0, [d] 2.67. Coadministration of CLG with HAL attenuated the development of the HAL induced SS only at dose [c]: (climbing scores induced by APO (1.0 mg/kg ip) went from +192% (IIa) down to +117% (IIc) of values in control mice [Ia]). The dose response curve for CLG is thus bell-shaped (highest doses not effective) and is similar to many other curves for MIF-1 & CLG effects. The ability to prevent a dopaminergic hypersensitivity induced by neuroleptics suggests a potential therapeutic use in the prevention of tardive dyskinesia. (Supported in part by the Tourette Syndrome Assn & VA grants to JZF and RFR)

375.10

CYCLO(LEUCYL-GLYCYL)(CLG) REVERSES THE PERMANENT DOPAMINERGIC SUPERSENSITIVITY INDUCED BY OVARIECTOMY. JH Gordon[1], JZ Fields[1], R Sami[1]*, JM Lee[1]*, & RF Ritzmann[2], Research Services, Hines & Brentwood VA Hospitals, Hines IL 60141 & Olive View/UCLA Med Ctr, Los Angeles CA 91342.

Several neuropsychiatric disorders (e.g. Tourettes, tardive dyskinesia, schizophrenia) are thought to involve a permanent dopamine (DA) receptor (DAr) supersensitivity (SS). Although many animal models of DAr SS have been studied, the only models in which the SS is permanent are induced by lesions and are obviously not appropriate for evaluating compensatory changes in the presynaptic DA neurons induced by post-synaptic DAr up-regulation. The ovariectomized (OVX) rat circumvents this problem as the the upregulated D2 DAr & the behavioral SS to DA agonists that develop (3 mos post-OVX) appear to be permanent while the DA neurons remain intact. CLG is a DA neuromodulator which prevents the development of a DAr SS in several animal models. However, it has never been shown whether CLG can reverse an already established behavioral SS to DA agonists. We now document such a reversal in the OVX model. OVX rats showed significantly more stereotypic sniffing after apomorphine (APO: 0.45 mg/kg, ip) & more locomotor activity (APO: 0.15 mg/kg). CLG (1X/day for 4 days, 8 mg/kg, sc) reversed these SS responses. We conclude [1] that the OVX rat is useful for modelling the permanent or irreversible aspect of DA SS; [2] CLG may be useful in the treatment of DA related diseases. (Supported by the Tourette Syndrome Assn & VA grants to JZF and RFR)

375.11

ATTENUATION OF SCH-23390-INDUCED ONTOGENIC IMPAIRMENT OF RAT STRIATAL DOPAMINE D-1 RECEPTORS BY L-PROLYL-L-LEUCYL-GLYCINAMIDE (PLG). R.M. Kostrzewa and M.I. Saleh*. Quillen-Dishner College of Medicine, East Tennessee State Univ., Johnson City, TN 37614.

While the effects of receptor antagonists on the development of striatal dopamine D-2 receptors have been studied by several groups, ontogenic actions of D-1 receptor antagonists have not been determined. Rats were treated from the day of birth and once each day for 32 successive days with the selective D-1 antagonist, SCH-23390 (0.30 mg/kg/d i.p.). Using the D-1 binding assay of Schulz et al. (J. Neurochem. 45: 1601, 1985), it was found that chronic SCH-23390 treatment during postnatal ontogeny resulted in a 70-80% reduction in binding of [^{3}H]SCH-23390 to striatal membranes in vitro at 5, 8 and 12 weeks from birth. These changes were associated with a 78% reduction in B_{max} and unchanged K_D, as assessed at 5 weeks. When 12 wk old rats with reduced numbers of striatal D-1 receptors were challenged by 17 d treatment with SCH-23390 (0.30 mg/kg/d i.p.), the D-1 receptors up-regulated 3-fold, up to 75% of control levels. When PLG (1.0 mg/kg/d i.p.) was co-administered with SCH-23390 during development, the ontogenic impairment of striatal D-1 receptors was totally attenuated. These findings demonstrate that the postnatal period is critical in the development of striatal D-1 receptors, and that PLG protects against the adverse ontogenic effects of SCH-23390.

375.12

ATTENUATION OF SPIROPERIDOL-INDUCED ONTOGENIC IMPAIRMENT OF RAT STRIATAL DOPAMINE D-2 RECEPTORS BY L-PROLYL-L-LEUCYL-GLYCINAMIDE (PLG). M.I. Saleh* and R.M. Kostrzewa (SPON: E.A. Daigneault). Quillen-Dishner College of Medicine, East Tennessee State Univ., Johnson City, TN 37614.

Postnatal treatment of rats for 3 weeks with haloperidol results in a persistent increase in the number of striatal dopamine D-2 receptors (Rosengarten and Friedhoff, Science 203: 1133, 1979). Because striatal D-2 receptor development occurs through at least the first 4 weeks from birth, it was of interest to determine the effect of prolonged treatment with a D-2 antagonist, spiroperidol (SP, 1.0 mg/kg/d i.p. 32d from birth), on development of D-2 receptors. Postnatal SP treatment reduced [^{3}H]SP binding to striatal homogenates by 50 to 75% at 5, 8 and 12 weeks from birth. The B_{max} for [^{3}H]SP binding was reduced by 74% at 5 weeks, while the K_D was unchanged. Co-administration of PLG (1.0 mg/kg/d i.p. x 32d) resulted in an attenuation of the D-2 ontogenic impairment produced by SP. These findings indicate that the postnatal period is a sensitive and critical one in regard to striatal D-2 receptor development and that PLG is able to attenuate SP-induced ontogenic impairment of D-2 receptors.

375.13

STRIATAL D-1 DOPAMINE RECEPTOR DENSITY FLUCTUATES DURING THE RAT ESTROUS CYCLE. D. Lévesque*, S. Gagnon* and T. Di Paolo, Department of Molecular Endocrinology, Laval University Medical Center, Quebec, G1V 4G2 and School of Pharmacy, Laval University, Quebec, G1K 7P4, CANADA.

Studies on dopamine (DA) content and turnover across the estrous cycle of rodent illustrate the importance of gonadal steroids in striatal DA regulation. In this study, we have investigated the binding characteristics of striatal D-1 DA antagonist binding sites in intact female, ovariectomized (OVX) animals and during the 4-day estrous cycle. Our results show that the affinity of the striatal D-1 receptor as labelled with [^{3}H]SCH23390 remains constant in female rats during the estrous cycle and OVX animals. By contrast the density of the striatal D-1 DA antagonist binding sites vary during the estrous cycle or after castration. Ovariectomy decreases striatal D-1 density by 16.8% ($p < 0.01$) compared to intact female rats. The density of striatal D-1 DA receptor was higher on the day of diestrus I (DI) and diestrus II (DII) ($p < 0.01$ vs OVX) and fluctuates throughout the estrous cycle with a maximum on the day of DII ($p < 0.05$ vs proestrus PM). The modulation under physiological conditions of striatal D-1 DA receptors as reported here is not correlated in a simple fashion with the variation of a single hormone and may involve several hormones acting synergistically or in opposite way as well as the delayed action of hormones may be observed. Interestingly, ovarian hormones seem to exert a stimulatory role on D-1 DA receptors density while we observe the opposite on the D-2 DA agonist affinity sites. Supported by the MRC.

CATECHOLAMINES IV

376.1

EFFECTS OF THE CATECHOLAMINERGIC NEUROTOXIN, DSP-4, ON ADRENAL CHROMAFFIN CELLS. P. Boksa, Douglas Hosp. Res. Ctr., Depts. Psychiatry and Pharmacology, McGill Univ., Montreal, Quebec, Canada H4H 1R3

N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) is an inhibitor of noradrenaline (NA) uptake and a neurotoxin known to deplete NA levels with little effect on dopamine (DA), serotonin or adrenaline (A) in the CNS. In the adrenal medulla, A, NA and probably DA are synthesized in separate chromaffin cells together with various neuropeptides; thus a neurotoxin targeting a specific sub-population of chromaffin cells would be a useful pharmacologic tool. The present study tested effects of DSP-4 on catecholamine uptake, release and content in cultured bovine adrenal chromaffin cells. DSP-4 selectively inhibited the acute uptake of [^{3}H]NA with little effect on [^{3}H]A or [^{3}H]DA uptake. In cultures pre-loaded with [^{3}H]catecholamines, DSP-4 stimulated release of [^{3}H]NA, and to a small extent also [^{3}H]A and [^{3}H]DA. However the drug did not stimulate the release of endogenous A, NA or DA. A high concentration of DSP-4 inhibited the carbachol-stimulated release of A, NA and DA. A 1 h exposure to DSP-4 decreased A, NA and DA levels with no gross morphologic changes in the cells. Reductions in A and NA levels were equal in magnitude, while DA was depleted to a somewhat greater extent under some conditions. Longer exposure to DSP-4 resulted in morphological changes in the cells suggesting that the drug is also toxic to chromaffin cells in culture. Supported by MRC of Canada.

376.2

D2 DOPAMINE RECEPTOR-MEDIATED INHIBITION OF CATECHOLAMINE SECRETION, ^{45}Ca UPTAKE AND CALCIUM CURRENTS IN BOVINE CHROMAFFIN CELLS. L. Bigornia*, C. Allen and A.S. Schneider Dept. Pharm. Tox., Albany Med. Coll., Albany, NY 12208.

D_2 dopamine receptors have been identified on chromaffin cell membranes and dopaminergic agonists have been shown to inhibit catecholamine release and ^{45}Ca uptake. In the present study, we examined the subtype of dopamine receptor involved in mediating dopaminergic inhibitory effects. The D_1 receptor agonist SKF 38393 did not inhibit basal or nicotine-evoked catecholamine secretion. The D_2 receptor agonist apomorphine inhibited nicotine- and KCl (60 mM)-evoked catecholamine secretion and this was reversed by D_2 receptor antagonists but not by the D_1 receptor antagonist SCH 23390. Similar effects were observed in parallel experiments examining KCl-evoked ^{45}Ca uptake. These observations suggest that dopaminergic inhibition of catecholamine secretion is mediated predominantly by a specific D_2 receptor subtype. To further examine the mechanism by which dopaminergic receptors modulate Ca^{2+} uptake, we used whole cell patch clamp electrophysiological techniques to directly monitor voltage-gated calcium channels in cultured chromaffin cells. We found that apomorphine reversibly reduced voltage-activated calcium currents and this effect was blocked by the D_2 receptor antagonist haloperidol, thus suggesting that voltage-gated calcium channels undergo inhibitory modulation by D_2 dopamine receptors in bovine adrenal chromaffin cell cultures.

376.3

PRESENCE OF DOPAMINE AUTORECEPTORS IN PC12 CELLS. N.D. Courtney*, A.C. Howlett and T.C. Westfall. Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Evidence exists for the presence of dopamine (DA) autoreceptors on adrenal chromaffin cells. DA agonists inhibited nicotine evoked catecholamine release from adrenal chromaffin cells and this inhibitory effect was reversed by DA antagonists (Artalego et al., J. Physiol. (Lond.) 362: 359, 1985). Since PC12 cells are derived from transformed chromaffin cells, these cells may also express DA autoreceptors and would make an ideal system to study the intracellular signals that are coupled to such receptors. PC12 cells were grown in DME with 10% heat-inactivated fetal calf serum and 5% heat-inactivated horse serum. Between 1-3 x 10^6 cells were plated on 35 mm wells 16 hr prior to the release experiments. Cells were incubated at 37°C with or without the drugs with Gey's balance salt solution for 2 min. Released DA was measured in the buffer and the cellular DA was measured after the lysis of cells with 10% perchloric acid. HPLC-EC was used for the detection of DA. K^+ (56 mM) was shown to produce marked increase in DA release over basal. This K^+-induced enhancement of DA release was attenuated by apomorphine (1 µM). Furthermore, haloperidol (10 µM) reversed the apomorphine-induced attenuation of K^+ stimulated DA release. Haloperidol (10 µM) had no effect on basal DA release. These results suggest the presence of DA autoreceptors on PC12 cells. (Supported by DA03690 and DA02668.)

376.4

BRADYKININ STIMULATES THE RELEASE OF CATECHOLAMINE FROM RAT PHEOCHROMOCYTOMA PC-12 CELLS. H. Houchi, J.S. Masserano* and N. Weiner. Dept. of Pharm., Univ. of Colo. Health Sci. Ctr. 4200 E. Ninth Ave. Denver CO 80262.

Bradykinin is a putative central nervous system neurotransmitter. In a number of cell systems, bradykinin has been shown to increase the accumulation of inositol trisphosphate (IPs). We have evaluated the effect of bradykinin on the release of endogenous dopamine from PC-12 cells. Bradykinin released dopamine in a concentration dependent manner with maximal release (15% of total stores) occurring at 10 µM. The release of dopamine by bradykinin occurred in two phases. The first phase occurred rapidly with 8% of total stores of dopamine released within 15 sec; the second phase was slower, with an additional 7% release occurring between 2 and 10 min. Ten seconds after bradykinin treatment intracellular Ca^{++} measured by Fura-2 was increased 600%. One minute after bradykinin treatment $^{45}Ca^{++}$ uptake was increased 100%. Ten minutes after bradykinin treatment, the formation of [^{3}H]-inositol phosphates were increased 360%. These data indicate that bradykinin regulates the release of dopamine from PC-12 cells, possibly by increasing IPs formation and by activating calcium/calmodulin-dependent protein kinase and protein kinase C. Supported by USPHS grants NS07927 and NS09199.

376.5

CATECHOLAMINE (CA) SECRETION MEDIATED BY A OUABAIN RECEPTOR IN THE BOVINE CHROMAFFIN CELL. C. Gonzalez-Garcia*, V. Cena*1 and H.R. Keiser* (SPON: I. De Andres). Hypertension-Endocrine Br., Nat. Heart, Lung, and Blood Institute, and [1]Lab. of Cell Biology and Genetics, Nat. Inst. of Diabetes, Digestive and Kidney Diseases, Bethesda, MD 20892.

We studied the secretion of CA from isolated bovine adrenal chromaffin cells in culture evoked by different cardiac glycosides, its correlation with blockade of Na^+, K^+-ATPase activity and the presence of the cardiac glycoside receptor as indicated by the presence of ^{3}H-ouabain binding sites. Cardiac glycosides release CA (up to l0% of total content) in a dose-dependent manner with an order of potency strophanthidin > ouabain > acetyldigitoxin > digitoxin. The ED_{50}'s varied from l μM for strophanthidin to l0 μM for digitoxin. ^{3}H-ouabain was specifically bound to a single population of high affinity binding sites located on the plasma membrane of chromaffin cells, with a K_d (l3.6 nM) similar to that found in other neural tissues, and a B_{max} of 3.l5 pmol/mg protein.^{3}H-ouabain was specifically displaced from this binding site by cardiac glycosides with an order of potency ouabain > acetyldigitoxin > digitoxin. The IC_{50}'s for displacement range between l0 nM for ouabain and 75 nM for digitoxin. These results indicate a discrepancy between the doses of cardiac glycosides required to occupy the receptor (likely Na^+,K^+-ATPase) and the doses that evoke CA secretion from chromaffin cells.

376.6

ESTROGEN PRIMING INCREASES STRIATAL DOPAMINE RESPONSIVENESS TO 60 mM KCl IN MALE RATS. B.L. Firestein* and J.B. Becker (SPON: S. Gilman). Psychology Dept. & Neuroscience Program, The University of Michigan, Ann Arbor, MI

Previous experiments have shown that there are sex differences in the effects of gonadal steroid hormones on behavioral and neurochemical indices of striatal dopamine (DA) activity. Development of these sex differences appears to be dependent on the presence of the ovaries around the time of puberty (Becker & Ramirez, Neuroendocrinology, 32:168-173, 1981). The experiments to be reported examine the role of estrogen during the peripubertal period of male rats on subsequent striatal DA responsiveness to estrogen.

Male rats 36 days old were castrated and treated with either estradiol benzoate (5 μg) or vehicle for a total of 12 days. After the last treatment, animals were killed by decapitation and striatal tissue was dissected, sliced and placed into superfusion chambers as described previously (Becker et al., J. Neurosci. Meth., 11:19, 1984). After baseline samples were collected, estradiol was infused in pulses [10 min. estradiol (E, 90 pg/ml); 20 min without E] for 3 pulses (90 min). Striata from half of the animals in each group received infusions of vehicle in pulses that paralelled the E pulses. Following the E pulses 60 mM KCl was infused for 2.5 min. Endogenous DA in the effluent was measured by HPLC-EC.

In striatal tissue from males pretreated with estrogen, pulsatile E in the superfusion medium potentiated the subsequent responsiveness to KCl depolarization, relative to the responses of control groups ($p<0.01$). The pulsatile infusion of E in the superfusion medium had no effect on the basal efflux of DA for either group. We conclude that estrogen pretreatment predisposes striatal DA neurons in prepubertal males to be responsive to subsequent estogen exposure. (BNS 84-11763).

376.7

PEPTIDE AND AUTORECEPTOR MODULATION OF DOPAMINE RELEASE FROM EXPLANTED MESOACCUMBENS (SOMA-TO-TERMINAL) TISSUE. M.D. Davis* and C.D. Kilts* (SPON: R.M. Quock). Dept. Biology, Univ. Wisconsin, P.O.B. 413, Milwaukee, WI 53201, and Dept. Psychiatry, Duke Univ., Durham, NC 27710.

Midbrain dopamine neurons exhibit several interesting characteristics including somatodendritic and terminal release/synthesis-regulating autoreceptors, and neurotransmitter release from somatodendritic as well as terminal regions. Here we report on certain dimensions of these properties in mesoaccumbens dopamine (DA) neurons utilizing the new technique of "core" explantation which isolates an elongated projection from cell bodies to their terminals.

Male Sprague-Dawley rats (14-21 days old), were decapitated, their brains removed and chilled in buffer. A sharpened cannula (0.6mm i.d.) was driven through the tissue block along a specified pathway, collecting ventral tegmental area (VTA) dopamine cell bodies, medial forebrain bundle (MFB) axons and nucleus accumbens terminals. The explant core was removed from the cannula and perifused in vitro. Media was collected at both ends of the explant for separate analysis of released DA via HPLC-EC.

Following VTA administration of neurotensin (0.06-6 uM), TRH (0.5-5 uM) and substance P (0.5-5 uM), a sustained increase (150-300%) in terminal DA release was observed, lasting for up to 30 minutes. Sulpiride (50 nM) augmented neurotensin-induced DA release, while the DA D2 antagonist, LY171555 (0.1 uM), attenuated it. Stimulation of the MFB evoked an autoreceptor-mediated decline in DA stores.

376.8

A POTENT DOPAMINE-RELEASING PROTEIN (DARP) IS PRESENT IN HIGH CONCENTRATIONS IN THE ADRENAL GLAND. G.D. CHANG AND V.D. RAMIREZ, DEPARTMENT OF PHYSIOLOGY AND BIOPHYSICS, UNIVERSITY OF ILLINOIS AT CHAMPAIGN-URBANA, URBANA, IL 61801.

A Hartree's procedure was adopted to preferentially extract the DARP from rat adrenal gland. The 80 % ethanol precipitate was then dissolved and dialyzed against a modified Kreb's Ringer Phosphate buffer (KRP) and used for the in vitro superfusion experiments. This adrenal dialysate elicited dose-dependent rapid and robust increases in endogenous dopamine (DA) release from the rat corpus striatum (CS) accompanied by similar changes in DOPAC release. In addition, the adrenal dialysate also induced small but significant increases in epinephrine output from the superfused adrenal medulla. Further characterization of the adrenal dialysate with Sephadex G-75 column chromatography suggested an apparent Mr 54,000 for the DARP. A similar DARP was purified from bovine adrenal gland to apparent homogeneity under alkaline and SDS PAGE analysis. SDS PAGE of the purified protein showed a single protein band of Mr 55,000. The bovine DARP induced significant increases in endogenous DA release from the rat CS at concentrations around 10^{-7}M.

376.9

GENETIC DIFFERENCES IN INHIBITION OF SYNAPTOSOMAL ^{3}H-MONOAMINE UPTAKE BY COCAINE, AMPHETAMINE AND TROPACOCAINE. J.A. Ruth, T.Z. Bosy* and C. C. Duncan. School of Pharmacy, University of Colorado, Boulder, CO 80309-0297.

We have shown that the BALB, C3H, C57 and DBA inbred strains of mice differ in behavioral sensitivity to cocaine (COC) as well as in sensitivity to COC inhibition of synaptosomal uptake of ^{3}H-NE, ^{3}H-DA and ^{3}H-5HT. To assess the generality of these differences for other inhibitors of monoamine uptake, the inhibition of accumulation of ^{3}H-NE, ^{3}H-DA and ^{3}H-5HT by d-amphetamine (AMPH) and tropacocaine (TROP) was examined in synaptosomes from each strain of mouse. For inhibition of ^{3}H-DA uptake, the relative order of potency observed was AMPH > COC > TROP. C57 was most sensitive to AMPH (IC_{50} = 10^{-8} M), and DBA least sensitive (IC_{50} = 10^{-7} M). AMPH was 5-10 fold more potent than COC, and 100 fold more potent than TROP. For inhibition of ^{3}H-5HT uptake, the order of potency observed was COC > AMPH = TROP. DBA was most sensitive to COC (IC_{50} = 8 x 10^{-8} M), and C57 least sensitive (IC50 = 10^{-6} M). COC potency ranged from 100 fold (DBA) to 10 fold (C57) greater than AMPH. For inhibition of NE uptake, AMPH and COC were equipotent in BALB and C57 (IC_{50} = 10^{-7} M), but COC was 10 fold less potent than AMPH in DBA and C3H. The drugs did not significantly differ in maximal lipid solubility in partition experiments. The data suggest genetic differences in the topology of respective monoamine carriers, and suggest that differences in behavioral response to stimulant drugs may involve complex monoamine interactions.

376.10

ROLE OF CORTICOSTRIATAL NERVE PROJECTIONS IN THE REGULATION OF BINDING SITES FOR DOPAMINE UPTAKE BLOCKERS IN RAT CAUDATE NUCLEUS. M. Grilli*, E. Sanna* and I. Hanbauer. HE Branch, NHLBI, N.I.H., Bethesda, MD 20892.

Ablation of the frontal and medial cortex leads to destruction of corticostriatal projections that are presumably glutamatergic/aspartergic in nature. These terminals were reported to impinge directly on dopaminergic terminals in caudate nucleus where they were shown to stimulate ^{3}H-dopamine release. We have studied the effect of cortical ablation on ^{3}H-dopamine uptake and on the regulation of Na^+-dependent specific binding of various radiolabelled dopamine uptake blockers. The ^{3}H-dopamine uptake in slices of caudate nuclei ipsilateral to the lesioned cortex was more sensitive to the inhibition by cocaine (K_i=2.6 x 10^{-7}M) or mazindol (K_i=7.5 x 10^{-7}M) than the contralateral side (K_i=1.3 x 10^{-6}M) three weeks after cortical ablation. In addition, the number of Na^+-dependent ^{3}H-cocaine binding sites in striatal membranes was increased (contralateral: 3.6 pmol/mg protein; ipsilateral: 8.0 pmol/mg protein). In contrast, the binding properties of ^{3}H-phencyclidine, ^{3}H-MK 801, and ^{3}H-GBR 12935, substances that also block dopamine uptake, remained unchanged. The number of ^{3}H-mazindol binding sites was increased by 20% in striatal membranes from the decorticated hemisphere. The present results show that in absence of corticofugal nerve endings the dopamine transport system in caudate nuclei is up-regulated through an allosteric mechanism. It is inferred that corticofugal nerve endings may release an endogenous modulator that inhibits dopamine uptake.

376.11

IN VIVO AUTORADIOGRAPHY OF [^{18}F]-GBR 13119 BINDING IN RAT BRAIN. M.R. Kilbourn*, B.J. Ciliax, M.S. Haka*, and J.B. Penney, Depts. of Internal Medicine and Neurology, University of Michigan, Ann Arbor, MI 48104.

We have examined [^{18}F]-GBR 13119's ability to bind to the dopamine uptake site in vivo in adult male Sprague-Dawley rats. Quantitative autoradiography was performed on coronal sections (20 μm) taken through neostriatum, the A9/A10 region, and cerebellum of rats injected with 0.5 - 1.5 mCi [^{18}F]-GBR 13119.

[^{18}F]-GBR 13119 binding was high in striatum, nucleus accumbens, and olfactory tubercle, moderate in ventral tegmental area and substantia nigra pars compacta, and low in cerebral cortex and cerebellum. [^{18}F]-GBR 13119 binding in ipsilateral striatum of unilateral 6-hydroxydopamine lesioned rats was reduced to background levels. Predosing with "cold" GBR 13119 (10 mg/kg) blocked binding throughout the brain, but predosing with fluoxetine (20 mg/kg) or nisoxetine (5 mg/kg), specific serotonergic and noradrenergic uptake inhibitors, respectively, did not block [^{18}F]-GBR 13119 binding. Using PET and [^{18}F]-GBR 13119, we have successfully imaged the striatum of a monkey with a striatum/cerebellum ratio of 1.8. [^{18}F]-GBR 13119 should be a useful presynaptic marker for dopamine neurons and could be used to PET scan patients with Parkinson's Disease.

Supported by USPHS grant NS 15655.

376.12

SOLUBILIZATION OF THE DOPAMINE TRANSPORTER. M.J. Kuhar, J. Sharkey* and D.E. Grigoriadis. (SPON: S.Bird). NIDA Addiction Research Center, Baltimore MD 21224

^{3}H-GBR12935 is recognized as a potent and highly selective ligand for the dopamine transporter, the site related to the reinforcing properties of cocaine. In the present study, ^{3}H-GBR12935 was used to label the solubilized dopamine transporter in digitonin treated striatal membranes. Homogenates of rat striata (50mg/ml) were solubilized in 1% digitonin for 30 min, centrifuged for 1 hr at 100,000g, the supernatant filtered and 0.3ml aliquots used in binding assays. ^{3}H-GBR12935 exhibited reversible, saturable, high affinity binding characteristics to solubilized membranes with an observed K_D of 4nM; a value similar to the 1nM K_D observed in striatal membranes. In competition studies, ^{3}H-GBR12935 binding to solubilized and to membrane homogenate preparations was inhibited in a similar fashion by the dopamine uptake blockers GBR12909, mazindol, nomifensine and benztropine but not by the 5HT and norepinephrine uptake blocker doxepin. Furthermore, following 6-hydroxydopamine lesions of the nigrostriatal pathway parallel reductions in ^{3}H-GBR12935 binding were observed in both homogenized and solubilized membrane preparations. These data indicate that the dopamine transporter can be solubilized and labeled with ^{3}H-GBR12935, providing a useful tool towards the molecular characterization and purification of the dopamine transporter.

376.13

SOLUBILIZATION OF THE DOPAMINE TRANSPORTER: F.R. Sallee*, R.L. Stiller*, H.B. Niznik, N.H. Bzowej, P. Seeman and J.M. Perel*. **Clinical Pharmacol. Prog. WPIC, Univ. of Pittsburgh, Pittsburgh, PA 15213 and Depts. of Medicine and Pharmacol. Univ. of Toronto, Toronto, Ont. M5S 1A8.**

The dopamine transporter, as indexed by [^{3}H]GBR-12935 binding, was solubilized from canine striatal membranes with the detergent digitonin. The protein retained the same pharmacological characteristics as membrane-bound uptake sites. The binding of [^{3}H]GBR-12935 to solubilized preparations was specific, saturable and reversible with a dissociation constant of ~3 nM and a site density of 3.4 pmol/mg protein. [^{3}H]GBR-12935 also bound to solubilized sites in a sodium-independent manner with a K_D of ~6 nM and a 60% decrease in site density. Dopamine uptake inhibitors and substrates inhibited [^{3}H]GBR-12935 binding in a stereoselective and concentration dependent manner with an appropriate rank order of potency for the dopamine transporter. K_D values for these compounds in solubilized preparations were virtually identical with those obtained on [^{3}H]GBR-12935 binding in the native state and correlated with IC_{50} values on [^{3}H]dopamine uptake. The dopamine transporter appears to be a transmembrane glycoprotein by virtue of its absorption and specific elution from WGA-lectin column. Solubilization of this protein with full retention of binding activity now allows for the purification and biochemical characterization of this important membrane protein.

CATECHOLAMINES: ELECTROPHYSIOLOGY II

377.1

ELECTROPHYSIOLOGICAL EFFECTS OF COCAINE IN THE RAT MEDIAL PREFRONTAL CORTEX. D.J. Henry and F.J. White. Dept. of Psychiat., Wayne St. Univ. Sch. Med. & Neuropsychopharm. Lab., Lafayette Clinic, Detroit, MI 48207.

Rats will readily self-administer cocaine directly into the medial prefrontal cortex (mPFC), indicating that this site may be important in mediating the rewarding effects of this popular drug of abuse. Surprisingly, little is known regarding the electrophysiological effects of cocaine in this area. In the present studies, extracellular single-cell recording and microiontophoretic techniques were employed to characterize the effects of cocaine in the dopaminergic terminal area within the mPFC (layers V & VI). Our studies indicate that both cocaine (0.01 M) and dopamine (0.1 M) exhibit a partial inhibitory effect (40-50% inhibition at 16-32 nanoampere current) on both spontaneously active and glutamate-stimulated mPFC cells recorded from male chloral hydrate-anesthetized rats. Procaine (0.01 M), administered in a similar fashion, exerted no such inhibitory effect. The inhibitory effects of cocaine and dopamine were blocked by co-iontophoresis of the D2 dopamine antagonist sulpiride (0.05 M, 16nA). At higher ejection currents, (>32 nA) local anesthetic-like effects (rate inhibition accompanied by spike amplitude reduction) were observed with both cocaine and procaine. Intravenous administration of cocaine also partially inhibited the firing of mPFC neurons. Present studies are investigating the mechanisms underlying the inhibitory effects of cocaine within the mPFC.

377.2

SINGLE UNIT ACTIVITY IN THE NUCLEUS ACCUMBENS OF THE FREELY MOVING RAT DURING ORAL SELF-ADMINISTRATION OF COCAINE. M. Wolske* and M.Q. West (SPON: C. Boast) Dept. of Psychology, Rutgers University, New Brunswick, NJ 08903.

The nucleus accumbens has been implicated as a critical neural substrate mediating psychomotor stimulant reinforcement. Previous electrophysiological studies of this region have been limited to anesthetized animals, in which results can be adversely affected by the anesthetic, and in which no behavioral assessment can be made regarding the reinforcing properties of drugs such as cocaine. These problems were eliminated in the present study, in which the schedule-induction technique was used to produce cocaine polydipsia (Tang, M. and Falk J., Pharmacol. Biochem. & Behav. 28:517, 1987). Male Long-Evans rats, aged 90-120 days, were prepared for chronic recording via a detachable, miniature microdrive positioned above the nucleus accumbens (A-P 2.2mm, M-L 1.5mm, D-V 5.8-8mm). Animals were food-deprived to 80% ad lib weight and conditioned to bar-press for food on a FI 60 schedule, in which a tone (1 kHz, 60 dB) served as a discriminative stimulus. Single unit recordings from the nucleus accumbens (verified on line via synaptic activation of the ipsilateral subicular input, and histologically after sacrifice) were analyzed in terms of peri-event histograms constructed around behavioral events such as tone onset, bar pressing and licking. Preliminary results suggest that, in contrast to anesthetized preparations, cocaine produced a general increase in the spontaneous activity of accumbens neurons. In particular, cocaine produced greater elevations in firing rate during movement (such as orienting and bar-pressing), similar to our previous study of caudate-putamen neurons (Shimizu et al, Neurosci. Abstr. 13:979, 1987). This enhancement was selective in that, prior to drug consumption, these same movements were not accompanied by elevations in firing rate. Since the likely mechanism of cocaine's action is to block reuptake of dopamine, a selective effect of cocaine during movement is consistent with a large literature correlating increased dopamine release with movement. Supported by DA 04551, PHS RR 07058-21, NSF BNS-8708523.

377.3

REDUCED DURATION OF NEURONAL RESPONSE TO DOPAMINE IN THE ANTERIOR CINGULATE CORTEX AFTER UPTAKE BLOCKADE. Mario Beauregard and André Ferron. Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Canada.

To investigate the effect of dopamine (DA) uptake blockade on neuronal responsiveness to this catecholamine, we compared the duration of the response to DA before and after the administration of GBR 12909 (DA uptake blocker, 500 ug/kg, i.v.). The duration of inhibition induced by iontophoretic applications of DA (40 nA x 40 s) was assessed by the RT90 method, i.e. by measuring the time (in s) between the end of DA application and the return to a neuronal firing rate equal to at least 90% of the pre-injection frequency. In control rats, the duration of DA responses were significantly enhanced by GBR 12909 in the prefrontal cortex (87 s vs 459 s), in the striatum (100 s vs 460 s) as well as in the nucleus accumbens (52 s vs 209 s), but markedly reduced in the anterior cingulate cortex (ACg) (576 s vs 155 s). Furthermore, DA depletions and/or deafferentations, using either -MPT or 6-OHDA, produced a decrease in the duration of the responses to DA (61 and 54 s, respectively) in the ACg, comparable to that observed after GBR administration. A possible explanation for the present results is that in the ACg, under normal circumstances, the released DA is transported back into the presynaptic terminals and in this way induces a further DA release, thus favouring a longer duration of DA effects. Such a positive feedback mechanism could play a key role in the regulation of the activity of ACg neurons by modulating the homeostatic control of DA release. This control mechanism of DA release may be a particular feature of the mesocortical dopaminergic fibers projecting to the ACg and thus be involved in higher integration cortical functions.

377.4

EFFECTS OF AMPHETAMINE ON SINGLE UNIT ACTIVITY IN THE OLFACTORY TUBERCLE. **Charles H.K. West and Richard P. Michael. Department of Psychiatry, Emory University School of Medicine, and Georgia Mental Health Institute, 1256 Briarcliff Road, Atlanta, GA 30306.**

A principal action of d-amphetamine (AMPH), believed to be critical for its behavioral effects, is enhancement of transmission in the midbrain dopaminergic systems. Electrophysiological studies focusing on the effects of AMPH on the spontaneous activity of neurons in dopamine terminal regions have revealed that effects depend on site. Both increases and decreases in activity were induced by AMPH in striatal neurons, but effects were predominantly inhibitory in nucleus accumbens neurons. Here, we report on the effects of AMPH on neurons in the olfactory tubercle, a somewhat neglected area although it receives heavy dopaminergic innervation. Single units were recorded in adult male rats anesthetized with chloral hydrate (400 mg/kg, ip). After records had stabilized, spontaneous activity was measured before and at 5 min intervals after injection of AMPH (0.25 mg/kg, iv). Of the 14 units tested with AMPH to date, activity was increased in 11 (78%), decreased in one and not changed in two. The increase in activity (mean = 57%) was maximal 15–20 min after injection and returned to control levels by 45 min. Preliminary tests with haloperidol blocked the effects of AMPH on unit activity, suggesting that dopamine is involved in these actions of AMPH. Differences between structures in regard to the neuronal response to AMPH (e.g., olfactory tubercle and nucleus accumbens) may help us understand the different behavioral effects of this drug.

(Supported by the Georgia Department of Human Resources.)

377.5

ENHANCED SENSITIVITY OF SUBSTANTIA NIGRA DOPAMINE (DA) CELL AUTORECEPTORS FOLLOWING PARTIAL DA DEPLETIONS IN RATS. Michele L. Pucak and Anthony A. Grace. Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA, 15260.

Studies have shown that 2 weeks after lesions causing greater than 85% depletion of striatal DA, there is development of striatal DA receptor supersensitivity. However, it has not yet been established whether the DA cell body autoreceptor also undergoes changes in sensitivity. The present study investigated this problem using single-unit recording techniques from identified substantia nigra DA cells in 6-hydroxydopamine lesioned and control rats. The relative sensitivity of lesioned and control rats was estimated by their response to systemic administration of apomorphine (APO). APO was given intravenously to rats in each group in dose-response fashion. Preliminary results suggest a slight but not significant trend toward subsensitivity soon after the lesion (six to ten days). However, six to eight weeks post-lesion, our preliminary results suggest a supersensitivity of DA cell autoreceptors, with 50% inhibition in DA cell firing rate occurring with 3.2 ug/kg APO in the lesioned rats, as compared to 8 ug/kg in control rats. We are currently examining this phenomenon after striatonigral lesions to test for any postsynaptic contribution to the changes seen after striatal lesions. (Supported by NS19608).

377.6

EFFECT OF ACUTE AND CHRONIC TREATMENT WITH SCH 23390 ON THE SPONTANEOUS ACTIVITY OF MIDBRAIN DOPAMINE NEURONS. E. Esposito* and B.S. Bunney (Spons: David J. Barker). Dept. of Psychiat., Yale Univ. Sch. Med., New Haven, CT.

In the albino rat, chronic treatment with typical antipsychotic drugs (APD) induces a marked decrease in the number of spontaneously active A9 and A10 dopamine (DA) neurons due to the development of depolarization block (DB). It has been shown that SCH 23390, a selective blocker of D-1 DA receptors, has a biochemical and behavioral profile consistent with potential antipsychotic activity. In the present study, we investigated the effect of acute and chronic administration of SCH 23390 on the spontaneous activity of DA neurons, both in the A9 and A10 areas, in order to elucidate the role of chronic blockade of D-1 DA receptors on the induction of the DB produced by APD. Two groups of rats were given an acute subcutaneous (s.c.) injection of SCH 23390 (0.5 and 1.0 mg/kg). In four separate chronic experiments, SCH 23390 was administered repeatedly for 21 days either s.c. (0.5 and 1 mg/kg) or orally (5 and 10 mg/kg). Active A9 and A10 DA neurons were counted by passing an electrode through a stereotaxically defined block of tissue that could be reproducibly located from animal to animal. No changes in the number of spontaneously active DA neurons were found either after acute or chronic treatment with SCH 23390. D-1 receptor blockade, therefore, would not appear to be involved in the induction of DB by typical APD.

377.7

D1/D2 DOPAMINE RECEPTOR INTERACTIONS IN THE NUCLEUS ACCUMBENS: THE ROLE OF cAMP IN ELECTROPHYSIOLOGICAL RESPONSES. P.A. Johansen and F.J. White. Dept. of Psychiat., Wayne St. Univ. Sch. Med. and Neuropsychopharmacol. Lab., Lafayette Clinic, Detroit, MI 48207.

We have previously reported that a sufficient level of D1 dopamine (DA) receptor stimulation is necessary for (enables) functional expression of postsynaptic D2 DA receptor stimulation. To explore further the nature of this enabling phenomenon, we used iontophoretic techniques to determine whether the D1 receptor responsible for enabling is coupled to adenylate cyclase (AC). Extracellular, single-unit recordings were obtained from the nucleus accumbens (NAc) of chloral hydrate anesthetized male rats. Dose-response curves for three D1 DA agonists exerting varying efficacies (≈24-70% efficacy as compared to DA) at stimulating cAMP formation were compared (SKF 75670, SKF 38393, SKF 81297). Preliminary results with these compounds indicate no differences in their ability to inhibit the firing of NAc neurons, suggesting that activation of AC may not be involved in the inhibitory effects of D1 agonists on NAc neurons, or that only a small activation (≤24%) is required to produce such effects. In additional studies, iontophoretic application of dibutryl-cAMP or 8-bromo-cAMP failed to potentiate (enable) quinpirole-induced inhibition of NAc neurons in DA-depleted rats. The present results suggest the possibility that the mechanism by which D1 receptor stimulation enables D2 mediated inhibition of NAc activity may not involve activation of AC.

377.8

WHOLE-CELL RECORDINGS FROM NEURONS ACUTELY ISOLATED FROM THE RAT SUBSTANTIA NIGRA ZONA COMPACTA N. L. Silva, C.M. Pechura, J. ffrench-Mullen, and J.L. Barker. Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892.

Neurons were acutely isolated from the substantia nigra zona compacta of 1-3 week old rats by enzymatic and mechanical dissociation according to the methods of Kay and Wong (J. Neurosci. Methods 16:227-238, 1986). This procedure yielded numerous ovoid and fusiform shaped cell bodies with 2-5 truncated processes. Their electrophysiological properties were investigated immediately following dissociation using whole-cell patch recordings at room temperature. Typically these neurons exhibited spontaneous single or burst firing with spike heights of 60-70 mV. Current clamp recordings revealed that these neurons maintain resting membrane potentials between -50-60 mV and input resistances of 400-600 MΩ. Following the application of TTX spiking was eliminated and evidence of an inward rectifier was observed. Interestingly, this conductance has not been observed in embryonic cultured dopamine neurons. A variety of inward and outward currents were examined under voltage clamp. Two sustained outward currents could be distinguished by their sensitivity to TEA or Co and Cd. A transient outward current which was inactivated at depolarized potentials, deinactivated at hyperpolarizing potentials and sensitive to 4-AP was also present. Two TTX insensitive inward currents which were sensitive to Cd could be distinguished as one was inactivated at more depolarized potentials (-40 mV) and was slowly inactivating over time. In some cells, CCK was observed to enhance an inward current. This peptide is known to modulate the dopamine autoreceptor *in vivo* and in tissue slice experiments.

Nigro-striatal neurons were also labelled *in vivo* by the retrograde transport of rhodamine microspheres. Investigation of cells isolated from these animals is currently in progress.

377.9

IDENTIFIED MESOACCUMBENS DOPAMINE NEURONS *IN VITRO*. S. Rayport, J. Monaco* and S. Sawasdikosol*. Depts. of Psychiatry and Anat. & Cell Biol., Ctr. for Neurobiol. & Behav., Columbia Univ. and NYS Psychiatric Inst., New York, NY 10032.

Dysfunction of mesolimbic dopamine neurons may underlie the schizophrenic psychosis. We have used retrograde labelling to identify these cells in midbrain cell cultures. The nucleus accumbens of neonatal rat pups was injected with rhodamine-coated latex microspheres (Katz *et al.*, 1984), the ventral tegmental area was isolated after 3 days, dissociated (Kay and Wong, 1986), the cells cultured, and studied after 1 week.

Microsphere-labelled cells *in vitro* had the morphology of dopamine cells; they were large, multipolar, and had eccentric nuclei (*cf.* Berger *et al.*, 1982). Tyrosine hydroxylase and catecholamine staining revealed that at least 85% of labelled cells were dopaminergic (*cf.* Swanson, 1982). Intrasomatic Lucifer Yellow injection showed that the cells had grown extensive processes, with varicosities likely to be presynaptic sites; often a single neighboring cell also fluoresced, suggestive of gap junctional connections. Microsphere-labelled cells showed a number of the properties characteristic of midbrain dopamine neurons: *(i)* broad action potentials, measuring 1.6 ± 0.2 msec, from peak to 1/e, *(ii)* hyperpolarizing afterpotentials, *(iii)* anomalous rectification, *(iv)* low-threshold depolarizations often triggering spike generation, and *(v)* responsiveness to exogenous dopamine. Adding physiological criteria, *living* dopamine neurons can be unequivocally identified. Studying synapses formed by these neurons should contribute to understanding of the function of central dopamine systems.

377.10

THE EFFECTS OF DOPAMINE ON CENTRAL AMYGDALA NEURONS, IN VITRO INTRACELLULAR RECORDING. M.C. Schiess, E. Asprodini* and P. Shinnick-Gallagher. Dept. Pharmacology, Univ. Texas Medical Branch, Galveston, TX 77550.

The central nucleus of the amygdala receives a vast amount of monoaminergic input and has one of the highest concentrations of dopamine (DA) in forebrain structures. Using 500 micron coronal slices from adult male rats and intracellular recording techniques, we characterized the effects of superfusing one micromolar DA and apomorphine (Apo) on the central amygdala neurons. DA hyperpolarized the membrane potential, decreased resistance and increased conductance in 41% of the cells tested (N=34) and 50% tested with Apo. The voltage-current (V-I) relationship between control and DA had an intercept at -110 mv suggesting hyperpolarization was mediated by an increase in potassium conductance. In another 40% of the neurons DA superfusion produced hyperpolarization (10/34) or no change (4/34) in resting membrane potential, increased conductance and decreased resistance (Apo 25%). The V-I relationship between control and DA did not intercept but rather displayed a parallel shift. In a small percentage of neurons (12%) DA depolarized the membrane and decreased conductance. According to their active properties there are two types of neurons in the central amygdala (N=100). The majority of neurons do not accommodate, approximately 15% accommodate despite stimulation with large cathodal pulses. Both neuronal types have prominent after-hyperpolarizations that are reduced by DA.

377.11

RESPONSE OF MEDIAL ZONA INCERTA NEURONS TO VENTROMEDIAL HYPOTHALAMUS (VMH) STIMULATION AND APPLICATION OF DOPAMINE: AN *IN VITRO* STUDY. M.J. Eaton and R.L. Moss Dept. of Physiol., Univ. Texas Southwestern Med. Ctr., Dallas, TX 75235

The zona incerta (ZI), located in the subthalamic region of the diencephalon, contains dopaminergic neurons which are believed to have autoreceptors. The ZI has been implicated in such diverse functions as drinking behavior, gonadotropin secretion and sexual behavior. Anatomical studies have shown afferent input into the medial ZI from the VMH, another brain region which plays a role in these functions. The present study was designed to investigate the relationship between the effect of afferent input from the VMH and the micropressure application of dopamine on the electrical activity of neurons in the medial ZI.

Transverse sections through the ZI were obtained from Sprague-Dawley female rats and maintained in a slice chamber with continuous perfusion of warmed, oxygenated artificial cerebrospinal fluid. Extracellular single cell recordings were made from 347 neurons located in the medial ZI. The effect of micropressure application of 0.1M dopamine HCL on the neuronal activity, as well as the orthodromic response to VMH stimulation, was determined for each neuron.

			Dopamine Response		
			None	+	-
Orthodromic (OD)	None	(n=264)	66%	3%	31%
Response type	OD+	(n=73)	44%	5%	51%
(# of neurons)	OD-	(n=10)	60%	10%	30%

These data indicate a large, predominantly excitatory (OD+), input from the VMH to the medial ZI. Many of the cells in this region are dopaminergic and are believed to be capable of autoregulation. The results show a large proportion of the cells which are orthodromically excited by stimulation are also inhibited by dopamine, suggesting that VMH neurons project to and synapse on dopaminergic neurons in the medial ZI. Supported by HD09988-V.

377.12

DOPAMINE AND HALOPERIDOL MODIFY LOW THRESHOLD CALCIUM SPIKE AND TRANSIENT CALCIUM CURRENT IN GUINEA PIG THALAMIC NEURONS *IN VITRO*. E. Geijo-Barrientos and R. Llinás (SPON: S. Simon) Dept Physiol. & Biophy. N.Y.U. Med. Ctr. NY, 10016

Recent neuropharmacological work has implicated thalamic dopaminergic receptors in the genesis of certain psychiatric conditions (Oke & Adams, Schiz. Bul.13:589,1987). We have investigated the effects of dopamine and Haloperidol, a dopamine antagonist, on the electroresponsive properties of Guinea Pig thalamic neurons *in vitro*. In particular,calcium-dependent spikes and voltage-dependent calcium currents were investigated in thalamic slices using current clamp and single electrode voltage clamp techniques. In this preparation DA, at concentrations of 200μM, increased the peak amplitude of the transient calcium current by 20-40%. Two other types of experiments have shed light regarding the mechanism of this effect. (1) Haloperidol, which is known to be a blocker of D2 dopamine receptors, decreased the peak amplitude of this current by 44 ± 7% at concentrations of 5-10μM. (2). Forskolin (which increases intracellular cAMP levels) also decreased the peak amplitude of this current by 42.5 ± 3.45% at concentrations of 10μM. These experiments show that the effect of the DA is not mediated via D1 receptors, which, by being coupled to adenyl-cyclase, should increase the intracellular levels of cAMP. The action of these drugs on the low threshold calcium conductance suggests that a class of psychotic events may be related to altered intrinsic electroresponsiveness in thalamic and other central neurons. Supported by NIH grant NS13742 and a Fellowship to E. G-B from the Generalidad Valenciana.

377.13

EFFECTS OF APAMIN ON THE MEMBRANE PROPERTIES OF PUTATIVE DOPAMINE-CONTAINING NEURONS IN VITRO. P.D. Shepard, and B.S. Bunney. Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Recent studies utilizing brain slice preparations have reported differences between the electrophysiological characteristics of putative dopamine (pDA)-containing neurons in vitro and those previously associated with identified cells in vivo, including a tendency for cells in the former group to exhibit an extremely regular discharge pattern. Recently, we reported that administration of apamin, a polypeptide neurotoxin, induced sustained irregular single spike and bursting activity identical to that observed to occur spontaneously in vivo (Shepard and Bunney, Soc. Neurosci., 13:909, 1987). In the present study, intracellular recording techniques were employed to characterize apamin-induced alterations in the membrane properties of pDA neurons in vitro. Bath application of apamin (1 μM) selectively blocked a prolonged post-spike afterhyperpolarization (AHP) which could be attributed to activation of a calcium-dependent potassium conductance (gK(Ca)). The toxin had no effect on spike repolarization but enhanced a shorter duration AHP which had been partially 'masked' by the apamin-sensitive component. Blockade of the slow component of the AHP was accompanied by an increase in repetitive firing during current injection and a reduction in the magnitude of the post-activational AHP. These results suggest that apamin-induced alterations in the spontaneous discharge pattern of pDA-containing neurons in vitro are attributable to inhibition of gK(Ca). Supported by USPHS Awards MH-28849, MH-25642, MH-14276, and the State of Connecticut.

378.1

WITHDRAWN

378.2

ASSOCIATION OF TWO PERTUSSIS TOXIN SENSITIVE G-PROTEINS WITH THE D_2-DOPAMINE RECEPTOR FROM BOVINE STRIATUM. Z. Elazar*, G. Siegel*, C. David*, H. Kanety* and S. Fuchs (SPON: A.S. Gordon). Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

In studying the signal transduction mechanism of the D_2 dopamine receptor it was demonstrated that the solubilized receptor exhibits high and low affinity states for dopaminergic agonists. Guanine nucleotides and pertussis toxin can convert the solubilized receptor from a high affinity state to a low one. A D_2 receptor preparation from bovine striatum, partially purified by affinity chromatography on a haloperidol adsorbent, exhibited agonist stimulated GTPase activity. [^{32}P]-ADP-ribosylation by pertussis toxin of this receptor preparation resulted in the specific labeling of two protein bands corresponding to molecular weights of 39 and 41 kDa, in SDS-PAGE. Association of these G-proteins with the receptor was specifically inhibited by $Gpp(NH)_p$. Proteolytic fragmentation, immunoprecipitation and immunoblot analysis of these G-proteins, indicated that the 41 and 39 kDa protein bands are analogous to brain Gi and Go respectively. These experiments demonstrate that two distinct pertussis toxin sensitive G-proteins are functionally associated with bovine striatum D_2-dopamine receptor.

378.3

STIMULATION OF RAT STRIATAL GTPase BY ERGOLINES IN VITRO. J.D.Turner*, P.Marten*, K.-P.Gerbling* and H. Schneider* (SPON: L. Turski).
Research Laboratories of Schering AG (Berlin/Bergkamen), Neuropsychopharmacology, Müllerstraße 170-178, 1000 Berlin 65, F.R.G.
Many ergolines, with pharmacologically defined DA agonistic or antagonistic properties behave atypically in ligand binding assays. Accordingly, functional assays are required to characterise the dopaminergic properties of these substances in vitro. An assay recently proposed as specific for D2 agonism is the DA-stimulated GTPase detec table in striatal membranes (Onali, P. and Olianas, M.C., Biochem. Pharmacol. 36, 2839, 1987). In the present study, we have investigated the effects of some ergolines with differing pharmacological activity in this assay. In rat striatal membrane preparations, DA stimulated a "high affinity" GTPase dose dependently. This stimulation could be blocked by 100 nM haloperidol, but was unaffected by 100 nM Sch23390. Lisuride and pergolide (full D_2 agonists) stimulated GTPase activity in a haloperidol-sensitive manner. Terguride (a partial D_2 agonist) and proterguride (also reportedly a full D_2 agonist) both elicited bell-shaped response curves. Whether these results reflect different modes of interaction with DA receptors or interaction with different receptors (e.g. adrenergic, serotonergic) is currently being investigated.

378.4

DOPAMINE (DA) AGONIST PROFILE AT SYNTHESIS MODULATING DA AUTORECEPTORS (AR) IN STRIATAL SLICES, D. Clark*[1], E.A. Novak*, B.N. Mathews*, R.S. Salah, and M.P. Galloway (SPON: D.M. Kuhn) [1]Univ. Reading, Reading, UK, Wayne State Univ. Sch. Med. & Lafayette Clinic, Detroit, MI 48207

Utilizing striatal brain slices, we have developed an *in vitro* model to study synthesis modulating DA ARs. For eg., Salah et al. (this meeting) used this preparation to demonstrate that stimulation of DA ARs directly modulates the phosphorylation of tyrosine hydroxylase. Using several DA agonists from distinct chemical classes, we found that potencies (EC_{50}'s) obtained *in vitro* generally parallel those obtained in other AR models. Indeed, compounds such as (-)-3PPP and TDHL, previously defined as partial AR agonists, exhibit typical agonist character under basal conditions whereas their partial agonist character becomes evident after increasing extracellular DA. Notable, however, are differences among agonists in their intrinsic activity, i.e., the maximum inhibition of basal synthesis. Catechol containing compounds such as APO, DA, di-OH-aminotetralins, and SKF-38393 produce >90% inhibition whereas ergolines such as quinpirole decrease basal rates by 50% maximum. Although direct inhibition of TH by catechols may contribute to the net effect, the mono-OH aminotetralins (±)-7- and 5-OH-DPAT also exhibit 80-90% inhibition. This characteristic, combined with well-defined stereochemistry of the OH-DPATs, makes these AR agonists useful to define the *in vitro* AR model. Support: USPHS DA-4120, MH-41227, Mich. Dept. Mental Health.

378.5

NIGROSTRIATAL DOPAMINE AUTORECEPTORS INHIBIT TRANSMITTER SYNTHESIS BY DECREASING THE PHOSPHORYLATION OF TYROSINE HYDROXYLASE. R.S. Salah, D.M. Kuhn and M.P. Galloway. Depts. Pharmacology and Psychiatry, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

The *in situ* activity and phosphorylation of tyrosine hydroxylase (TH) was studied in rat striatal slices. TH activity was monitored by measuring the accumulation of dihydroxyphenylalanine (DOPA) in the presence of a decarboxylase inhibitor. TH phosphorylation was ascertained by SDS-PAGE and autoradiography after immunoprecipitation of TH with rabbit anti-TH serum. Incubation of slices in the presence of K^+ (55 mM), forskolin (10 uM), dibutyryl-cAMP (1 mM), or 8-bromo cGMP (100 uM) results in an increase in the activity and phosphorylation of TH. The dopamine (DA) autoreceptor agonist pergolide (3-10 uM) inhibits both basal and K^+-stimulated phosphorylation of TH. This change is accompanied by parallel changes in TH activity. The effect of pergolide is blocked by the selective D_2 antagonist eticlopride. In slices prepared from rats pretreated with intrastriatal injections of pertussis toxin, the inhibitory effect of pergolide was greatly diminished. These results are consistent with the hypotheses that 1) nerve terminal DA autoreceptors in the rat striatum are negatively coupled to adenylate cyclase, and 2) inhibitory effects of DA autoreceptor agonists on TH activity are due to a decrease in cAMP-mediated phosphorylation. Supported by MH 09673 (RSS), DA 04120 (MPG), and the State of Michigan.

378.6

STIMULATION OF DOPAMINE (DA) AUTORECEPTORS BY EMD 49,980. K. Harada[1]*, H. Yokoo[1]*, K. Tsuda[1]*, M. Goldstein[1] and E. Meller[2]. N.Y. Univ. Med. Ctr., New York, NY 10016, Neurochem. Res. Labs[1] and Millhauser Labs[2].

We have previously reported that EMD 23,448 has a selective DA action on presynaptic and supersensitive postsynaptic DA receptors (Goldstein et al., J. Neural Transm. 70:193, 1987). As a continuation of our studies we have examined the effects of EMD 49,980, a 5-hydroxy derivative of EMD 23,448, on DA autoreceptors regulating synthesis and release of DA. EMD 49,980 dose-dependently (0.01-0.5 mg/kg, i.p.) inhibited γ-butyrolactone-enhanced striatal dopa synthesis. Following partial inactivation of DA receptors with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (2 mg/kg) the IC_{50} for EMD 49,980 was shifted to the right, suggesting the presence of a receptor reserve at the DA autoreceptors. The electrically stimulated release of ^{3}H-DA from striatal slices was inhibited by EMD 49,980 at concentrations of 0.01-1uM. However, in the presence of the DA uptake inhibitor nomifensine (10 uM), EMD 49,980 did not inhibit the electrically stimulated release of ^{3}H-DA. The increased concentration of DA in the synapse in presence of nomifensine may diminish the DA agonist activity of EMD 49,980. The results of this study show that EMD 49,980 is a partial DA agonist whose potency is dependent on the synaptic concentration of DA. Supported by NIH grants NS-06801-21 and NS 23618.

378.7

COMPARISON OF RECEPTOR RESERVE AT DOPAMINE (DA) AUTORECEPTORS IN RAT STRIATUM, NUCLEUS ACCUMBENS AND OLFACTORY TUBERCLE. K. Bohmaker,* T. Puza,* M. Goldstein and E. Meller (SPON: J.C. Miller). Dept. of Psychiatry, NYU Medical Center, New York, NY 10016.

D2 DA autoreceptors in rat striatum which mediate local negative feedback inhibition of neurotransmitter synthesis show a large receptor reserve for N-propylnorapomorphine (NPA) (Meller et al., Mol. Pharmacol. 31:592-598, 1987), whereas postsynaptic striatal D2 receptors do not (Meller et al., this meeting). It was therefore of interest to determine whether DA autoreceptors in other brain regions (e.g. mesolimbic areas) also possess spare receptors for the full agonist NPA. Dose-response curves were obtained for NPA reversal of γ-butyrolactone (GBL)-induced L-DOPA accumulation in control and EEDQ (6 mg/kg)-treated rats. In control rats, the ED_{50}'s for NPA were 1.24, 0.44 and 0.34 ug/kg in striatum (STR), nucleus accumbens (NAS) and olfactory tubercle (OT), respectively. In EEDQ-treated rats the corresponding values were 3.36, 1.44 and 3.65 ug/kg. After EEDQ treatment, NPA maximally reversed the GBL-induced increase in L-DOPA levels by 57, 79 and 100% in STR, NAS and OT, respectively. Analysis of the results indicated that the DA autoreceptors in all three areas display non-linear receptor occupancy vs. response relationships for NPA; mesolimbic autoreceptors appear to have an even larger receptor reserve than striatal autoreceptors. Supported in part by PHS grants NS 23618, MH 02717 and MH 35976.

378.8

SCH 39166 HCl: A SPECIFIC D_1 RECEPTOR ANTAGONIST WITH ANTI-PSYCHOTIC POTENTIAL. L.C. Iorio, V. Ruperto*, M. Grzelak*, V. Coffin*, R.E. Chipkin and A. Barnett, Schering Corp., Dept. Pharmacology, 60 Orange Street, Bloomfield, NJ 07003, USA

This report is on a new D_1 antagonist, SCH 39166 (-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5H-benzo[d]-naphtho-[2,1b]-azepine-HCl (SCH). In vitro, SCH potently blocked D_1 receptors (Ki=2nM) and DA-stimulated adenylate cyclase activity (Ki=9nM). SCH was 250 fold less potent at D_2 receptors (Ki=512nM) and 75 fold less potent at 5HT2 receptors (Ki=151nM). In vivo, SCH inhibited conditioned avoidance responding in both rats (MED=10 po) and squirrel monkeys (MED=5 po) with a long duration of action; this is indicative of potential anti-psychotic activity. These in vivo effects are probably not due to D2 receptor blockade since SCH did not cause hyperprolactinemia in rats and did not block apomorphine-induced emesis in dogs. SCH increased dopamine turnover but only to levels 50-70% greater than baseline; in contrast, haloperidol increased levels by >300%. In summary, SCH is a specific D_1 antagonist both in vitro and in vivo with potent, long-lasting anti-CAR effects and is a potential novel anti-psychotic.

378.9

CHARACTERIZATION OF THE BINDING OF SCH 39166 TO D-1 RECEPTORS IN VIVO. R.D.McQuade, R.A.Harney* & R.E.Chipkin. Schering Corp., Bloomfield NJ 07003.

SCH39166 ([-]trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5H-benzo[d]naphtho-{2,1b}azepine), a new D-1 selective antagonist, has been analyzed for binding to D-1 receptors, in vivo. Rats receive a single s.c. injection of ^{125}I-SCH38840 (SCH23982) in the presence of increasing doses of SCH39166; after 1 h, the striata and cerebellum are removed and counted. SCH39166 inhibits striatal specific binding of ^{125}I-SCH38840, with an IC_{50} of 11.4 ± 3.9 nmoles. This value is nearly 4 x higher than that observed for SCH23390 (3 nmoles) and correlates well with the relative affinities of the molecules in vitro. However, SCH23390 inhibits greater than 90% of the striatal binding of ^{125}I-SCH38840, whereas 1 umole of SCH39166 displaces only 75% of the bound ligand. The remaining binding is not affected by higher doses of SCH39166 or by 3 nmoles of SCH23390, but 10 umoles of ketanserin, a 5HT-2 antagonist, significantly decreases the residual binding of ^{125}I-SCH38840. Thus, SCH39166, unlike SCH 23390, does not interact with 5HT-2 receptors in vivo. This study shows that SCH39166 selectively labels striatal D-1 receptors in vivo.

378.10

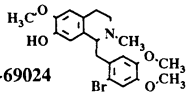
THE SELECTIVE D1 ANTAGONIST, SCH 23390 (SCH) DOES NOT INDUCE EXTRAPYRAMIDAL SIDE EFFECTS (EPS) IN CEBUS MONKEYS. VL Coffin*, MB Latranyi* and RE Chipkin, Schering Corp., 60 Orange St., Bloomfield, NJ 07003.

The following study evaluated the EPS liability of a D_1 antagonist SCH vs the predominantly D_2 selective antagonist, haloperidol (HAL). Cebus monkeys were given either vehicle (N=6), SCH (10 mg/kg po, N=9) or HAL (0.3 mg/kg po N=9) once a week. A Primate Observational Scale was developed and used to measure changes from normal behavior as well as occurrence of EPS. Both drug treatments produced significant increases in sedation scores which were equivalent in magnitude. Through 25 weeks of dosing, monkeys receiving SCH did not show any EPS. In contrast, by week 3, the HAL monkeys were significantly different from the VEH and SCH groups for EPS, and by week 13 all HAL monkeys showed severe EPS. In agreement with prior work, these animals are defined as 'primed' monkeys and also show EPS for the highly selective D2 antagonist, sulpiride (30 po), and for the classic neuroleptic, chlorpromazine (3.2 po). No EPS was seen when SCH (10 po) or the atypical neuroleptic, clozapine (20 po), were given to these recently 'primed' monkeys. These data confirm previous observations of D_2-induced EPS in cebus monkeys, and further demonstrate D_1 antagonists like SCH are unlikely to produce these side-effects.

378.11

A-69024 A NON-BENZAZEPINE D-1 SELECTIVE ANTAGONIST. D. J. Kerkman*, M. Ackerman*, L. D. Artman*, K. Asin, L. Bednarz*, M. C. Johnson*, R. G. MacKenzie, W. Montana, H. Stampfli* and J. W. Kebabian. Neuroscience Research Division, Pharmaceutical Discovery, Dept. 47U, Abbott Laboratories, IL 60046.

We report that A-69024 is an antagonist of the D-1, but not the D-2, dopamine receptor. Radioligand binding studies using rat striatum shows A-69024 (HBr) to have an apparent affinity toward the D-1 receptor of 12.9 *n*M (identified using [^{125}I]-SCH 23390), and an apparent affinity toward the D-2 receptor > 10 μM (identified using [^{125}I]-*N-p*-aminophenethylspiperone). In functional assays, A-69024 (HBr) is an antagonist of the D-1 receptor in fish retina (K_i = 48.3 *n*M) and the D-2 receptor in rat intermediate lobe (K_i = 728 *n*M); A-69024 (HCl) is an antagonist of rat striatum D-1 receptor (K_i = 12.3 *n*M). A-69024 (HBr), at a dose of 5 mg/kg *sc*, blocks amphetamine-induced locomotor activity and stereotypy, and SKF 38393- (but not LY 171555-) induced rotation in 6-OHDA lesioned animals; however, at this dose the compound does not increase serum prolactin levels or potentiate DOPA accumulation in rats pretreated with the DOPA decarboxylase inhibitor NSD 1015. Thus, A-69024 can discriminate between the D-1 and D-2 dopamine receptors and, therefore, may be a useful research tool complementing observations made with other D-1 agonists and antagonists.

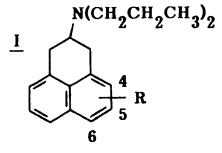


A-69024

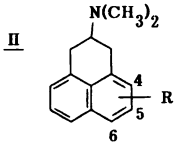
378.12

DOPAMINE AGONIST AND ANTAGONIST ACTIVITIES OF A SERIES OF SUBSTITUTED DIHYDROPHENALENES. A.H.Tang, S.R.Franklin, R.A.Code*, W.H.Darlington*, and J.Szmuszkovicz*. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

U-66444B (Ic) was reported to have presynaptic dopamine agonist activities (VonVoigtlander et al., 1987). Some closely related analogs are found to be either potent dopamine agonists or antagonists. The following compounds are emetic in dogs: Ia, Ic, Id, IIc, and IId. IIa and IIb are not emetic, but block apomorphine (Apo)-induced emesis. In rats trained to avoid shock in a shuttle box, Ia, Ic, Id, and IIc facilitate avoidance and increase movements between trials. IIa and IIb block avoidance and antagonize Apo-induced stereotypy. Neither compound produces catalepsy. In rhesus monkeys trained to discriminate 0.1 mg/kg of Apo from saline, both compounds block the discriminative stimulus effect of Apo. The dopamine agonist/antagonist activities in this series, therefore, depend critically on the position of the hydroxy group and the amino substitutions.



R = H, Ia, IIa
R = 5-OH, Ic, IIc



R = 4-OH, Ib, IIb
R = 6-OH, Id, IId

378.13

NO-112, NO-756 - NEW DOPAMINE D1 SELECTIVE ANTAGONISTS

Peter H. Andersen†, F.C. Grønvald°*, R. Hohlweg°*, L.B. Hansen°*, E. Guddal°* and C. Braestrup†*.
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NO-112 and NO-756 are new benzazepine derivatives. In vitro, NO-112 and NO-756 exhibit a K_i for the D1 receptor of 0.2 nM and inhibit dopamine-stimulated adenylate cyclase with a K_i of 2.5 nM. Corresponding values for SCH 23390 were 0.2 and 40 nM, respectively. NO-112 and NO-756 have also high affinity for the 5-HT_2 receptor (K_i-values of 18 and 4 nM, respectively). The iodina-ted analog of NO-756, i.e. NO-673, had similar affinity for both D1 and 5-HT_2 receptors (K_i=3.6 and 7.2 nM, respectively). In accordance, ^{125}I-NO-673 labelled in vitro and in vivo both D1 and 5-HT_2 receptors.

In conclusion, NO-112 and NO-756 are members of a new series of benzazepines with high affinity for the D1 receptor, but as compared to SCH 23390 with relatively higher affinity for the 5-HT_2 receptor and dopamine-stimulated adenylate cyclase. The latter characteristics equivalate to some extent those of clozapine i.e. NO-112 and NO-756 may have atypical neuroleptic characteristics like clozapine.

378.14

NO-112 AND NO-756, NEW POTENT BENZAZEPINE D-1 ANTAGONISTS: PHARMACOLOGICAL CHARACTERIZATION

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NO-112 and NO-756 ((+)-8-chloro-7-hydroxy-3-methyl-5(7-benzofuranyl)-2,3,4,5,tetrahydro-1H-3-benzazepin and (+)-8-chloro-7-hydroxy-3-methyl-5(7-dihydrobenzofuranyl)-2,3,4,5, tetrahydro-1H-3-benzazepin) are new selective D-1 receptor antagonists (see Andersen, P.H. et al., this meeting). The drugs inhibited amphetamine-induced discriminative effects in very low doses (0.02 mg/kg) and also blocked methyl-phenidate or amphetamine-induced stereotyped behavior, conditioned avoidance responding and D-1 receptor mediated rotational behavior in the same low doses. These effects were obtained at lower D-1 receptor occupancy in vivo when compared with the occupation of D-2 receptors required for obtaining the same effects. When coupled with a large body of data implicating a potential role for D-1 receptors in controlling psychosis, the profile of NO-112 and NO-756 suggests that they may exert anti-psychotic action in the clinic.

378.15

ELECTROPHYSIOLOGICAL AND METABOLIC EFFECTS OF (+)-AJ 76, A SELECTIVE ANTAGONIST OF DOPAMINE AUTORECEPTORS. W.E.Hoffmann, J.T.Lum, and M.F.Piercey, The Upjohn Company, Kalamazoo, MI 49001.

Svensson et al. report that (+)-AJ 76 and (+)-UH 232 are selective antagonists of dopamine (DA) autoreceptors (Naunyn Schmeideberg 334:234, 1986). We describe here 1) the antagonism of DA autoreceptor stimulation in substantia nigra pars compacta (SNPC), and 2) the use of 2-deoxyglucose (2-DG) autoradiography to map the neuroanatomical distribution of (+)-AJ 76 effects. DA neurons of chloral hydrate anesthetized rats were depressed by stimulation of autoreceptors with 100 ug/kg apomorphine (APO). (+)-AJ 76 and (+)-UH 232 reversed APO effects with ED50's of 480±233 and 103±53 ug/kg, respectively. The greater potency for (+)-UH 232 as an autoreceptor antagonist contrasts with its weaker stimulant effects (Svensson et al., ibid) suggesting that, compared to (+)-AJ 76, it is a less specific antagonist for autoreceptors. Pretreatment with either antagonist elevated APO's ED50. (+)-AJ 76 did not alter firing rates of 5-HT neurons in dorsal raphe. Using standard 2-DG protocol (Sokoloff et al., J. Neurochem. 28:897, 1977), 15 mg/kg i.v. (+)-AJ 76 injected 5 min prior to 2-DG increased glucose metabolism in SNPC, VTA, globus pallidus, and n. accumbens, sites also stimulated by dopamine agonists. Locus coeruleus metabolism was also increased. Like dopamine postsynaptic antagonist antipsychotics, (+)-AJ 76 stimulated the lateral habenula. It is concluded that (+)-AJ 76 is a selective DA autoreceptor antagonist.

RESPIRATORY REGULATION III

379.1

DEVELOPMENT OF A THICK MEDULLARY SLICE PREPARATION FOR THE STUDY OF RESPIRATORY RHYTHM GENERATION. H. McLean and J. Remmers, Departments of Medicine and Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

The location of neurons critical to the generation and maintenance of respiratory rhythm is unknown. We have developed a preparation from the brainstem of the neonatal rat (1-3 days old) in which diffusion distances are considerably reduced but respiratory activity and chemosensitivity are maintained. The preparation consists of a thick slice of brainstem extending 1 mm rostral and 1 mm caudal to the obex. Respiratory activity is recorded from the rostral hypoglossal nerve roots. The preparation is maintained at 32°C, superfused with mock CSF equilibrated with 95% O_2 and 5% CO_2 and is viable for approximately 4 hours. The thick slice preparation demonstrates more rapid and pronounced frequency responses to alterations in superfusate PCO_2 than the "intact" in vitro brainstem-spinal cord preparation, suggesting better diffusive accessibility of CO_2 to the central chemoreceptor. Preliminary experiments indicate that ventral regions of the medulla contain neural elements adequate for rhythm generation and chemosensitivity. In addition, when the rostral aspect of the medulla is removed, the frequency of respiratory rhythm is decreased suggesting that this area contains a frequency promoting region. This preparation presents a potentially useful model for investigation of the neural substrate for respiratory rhythm generation.

Supported by grants MRC #MA-9719 and CFSID.

379.2

LOCALIZATION OF A MEDULLARY AREA SENSITIVE TO CHANGES IN CO_2 IN IN VITRO BRAINSTEM-SPINAL CORD PREPARATION OF NEWBORN RATS. F. Issa* and J. Remmers. Dept of Medicine, Univ of Calgary, Calgary, Alberta, Canada T2N 4N1.

The exact location of the central chemoreceptor has not yet been determined. The in vitro brainstem-spinal cord preparation of 1-3 day old rats was used to identify areas within the medulla sensitive to changes in CO_2. The preparation was superfused with a mock CSF solution equilibrated with 5% CO_2-95% O_2 and kept at 28±1°C. A specially constructed compound glass micropipette with a tip diameter of 12-15 μm was used. The micropipette was filled with a mock CSF solution which was equilibrated with 100% CO_2. It was advanced in 50 μm steps into the ventral surface of the medulla using a stepping microdrive. At each depth, pressure ejection of 2-10 nl of CO_2-enriched mock CSF solution was performed while the inspiratory activity of C2-C4 ventral rootlets was continuously monitored. Slowing of breathing from a control of 10-15 to 1-5 bursts/min was observed with ejections made at depths of 0-200 μm in the area bound by hypoglossal nerve rootlets. An immediate and profound increase in breathing rate and amplitude of integrated C2-C4 neural activity occurred with injections in an area 0.50-0.75 mm lateral to midline, 0.5-0.8 mm rostral to the obex and at a depth of 0.25-0.35 mm from the ventral surface of the medulla. We conclude that neuronal elements in the medulla rostral to the obex, sense a change in PCO_2 and augment breathing.

Supported by grants MRC #MA-9719 and AHFMR #8731.

379.3

INTERACTIONS BETWEEN THE A-CURRENT AND CALCIUM CURRENT IN BULBOSPINAL NEURONS IN THE VENTRAL PART OF THE NUCLEUS TRACTUS SOLITARIUS. M.S. Dekin, T.H. Morgan School of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506

The Dorsal Respiratory Group in guinea pigs is located within the ventral part of the nucleus tractus solitarius (ventral NTS). Two classes of bulbospinal neurons, termed types I and II, have been identified within the ventral NTS. Both classes of cells express A-current (I_{KA}) and calcium current (I_C). During depolarization, interactions between I_C and I_{KA} will affect repetitive firing activity. In type I neurons, this interaction is manifest as a low frequency burst of spike activity while in type II neurons a long delay is seen between the onset of depolarization and the beginning spike activity. Using brainstem slices from adult guinea pigs and the single electrode voltage clamp technique, the contribution of I_C and I_{KA} to these repetitive firing patterns was studied. In type I neurons I_{KA} was activated at membrane potentials below spike threshold. In type II neurons I_{KA} was activated at membrane potentials which straddled spike threshold. I_C in type I neurons was activated at membrane potentials just below spike threshold while in type II neurons I_C was only observed at membrane potentials more positive than threshold. These data show that I_{KA} and I_C in type I neurons will compete for control of the membrane potential at spike threshold. In type II neurons I_{KA} would be activated at threshold without I_C, allowing it to dominate the membrane potential. (Supported by NIH Grant HL-39929).

379.4

***IN VITRO* INTRACELLULAR RECORDINGS IN RAT NUCLEUS TRACTUS SOLITARII AND NUCLEUS AMBIGUUS DURING HYPERCAPNIA.** **J.B. Dean, W.L. Lawing* and D.E. Millhorn. Dept. of Physiology and Curriculum in Neurobiology, UNC, Chapel Hill, NC 27599.**

In vitro intracellular recordings from cells in two respiratory-related areas, nucleus tractus solitarii(NTS) and nucleus ambiguus (NA), were made before and during exposure to hypercapnia(7, 10 and 15%CO_2). Intact & "dorsal" coronal slices prepared from rat were maintained in a humidified interface chamber(5%CO_2, 35-37°C). Most cells in NTS were unaffected or slightly hyperpolarized by CO_2. However, some NTS cells were depolarized and increased their activity during CO_2 in both regular and high Mg^{2+}/low Ca^{2+} synaptic blockade media. In some cases concomitant increases in input resistance were noted during CO_2-induced depolarization. This suggests the depolarization is due to a decreased K^+ conductance. Cells in NA were either hyperpolarized or unaffected by CO_2. Our findings show that a subpopulation of NTS cells are depolarized by CO_2/H^+. Although we cannot guarantee the specific function of these cells, we believe they may be involved in respiratory CO_2/H^+ chemoreception. (USPHS Grant HL33831 and NINCDS Training Grant 5-T32-NSO7166-08.)

379.5

CHARACTERISTICS OF POST-INHIBITORY REBOUND IN NEURONS WITHIN THE NUCLEUS AMBIGUUS OF ADULT GUINEA PIGS. S.M. Johnson* and P.A. Getting, Department of Physiology and Biophysics, University of Iowa, Iowa 52242

We are interested in the intrinsic properties of neurons that participate in the formation of the respiratory motor pattern in mammals. The Nucleus Ambiguus (NA) contains a concentration of heterogenous respiratory-related neurons including bulbospinal neurons, propriobulbar neurons, and vagal and glossopharyngeal motor neurons. The intrinsic properties of NA neurons were assessed by recording intracellularly (N= 150 cells) from transverse brainstem slices (350-400 microns thick, 0.5-1.5 mm rostral to the obex) of adult guinea pigs.

Following a 200-500 msec hyperpolarizing prepulse, post-inhibitory rebound (PIR) was expressed as a transient depolarization that could give rise to a spike or burst of spikes. PIR was observed in > 50% of the neurons. In the presence of TTX (0.5 ug/ml), PIR had a magnitude of 7-12 mv, a time to peak of 15-35 msec, and a duration of 150-200 msec. The magnitude of PIR depended on both the prepulse voltage and the test voltage (i.e. voltage to which the cell was released). The magnitude of PIR increased linearly as the prepulse voltage was made more negative (approx. 0.25 mv PIR/mv of hyperpolarization). At a constant prepulse voltage, the magnitude of PIR increased as the test voltage was made more positive (approx. 0.45 mv PIR/mv depolarization) until a maximum was reached at -55 to -60 mv.

The expression of PIR was enhanced by thyrotropin-releasing hormone (TRH, 100 nM). Under voltage clamp, however, the current underlying PIR was not affected by TRH. TRH modulation, therefore, must be mediated indirectly by depolarizing the cells or by decreasing the membrane conductance.

Supported by NIH grants NS15350 and HL32336 to PAG and a Lutheran Brotherhood Scholarship to SMJ.

379.6

SEROTONIN IMMUNOREACTIVE BOUTONS MAKE CLOSE CONTACTS WITH FELINE PHRENIC MOTONEURONS. J.Lipski*, P.M.Pilowsky*, M.D.Voss* and D.de Castro*. (SPON: R.Milne) Department of Physiology, University of Auckland, New Zealand.

There is a large body of evidence indicating that serotonergic mechanisms are involved in the central control of respiration. It has been suggested that this involvement only occurs indirectly, through an action by serotonin-containing neurons onto medullary respiratory neurons. In the present study we have investigated the possibility of a direct input by serotonergic neurons onto phrenic motorneurons.

Cats were anaesthetized with nembutal, and prepared for intracellular recording from antidromically identified phrenic motorneurons. Neurons were filled with Horseradish Peroxidase (10%, 2-10nA, 5-15min). After intravascular fixation and sectioning, filled neurons were detected with the diaminobenzidine-nickel reaction. The sections were then stained to reveal serotonin-like immunoreactivity using the Avidin-Biotin technique and the diaminobenzidine-imidazole reaction,. Phrenic motorneurons were well stained with the procedure used, and showed a dendritic arborization that extended for two to three millimetres rostrocaudally. Numerous serotonin immunoreactive boutons were found to make close contacts with identified phrenic motorneurons. These contacts were generally found on dendrites, and only occasionally on cell bodies. The results support the idea that serotonergic neurons may act directly on phrenic motorneurons.

379.7

SEROTONIN IMMUNOREACTIVE TERMINALS ARE INVOLVED IN SYNAPTIC CIRCUITS WITH PHRENIC MOTONEURONS. J.R. Holtman Jr. and B.E. Maley, Dept. of Pharmacology and Dept. of Anatomy and Neurobiology, Univ. KY Med. Ctr., Lexington, KY 40536

A number of neurotransmitters, including serotonin, are presumed to play a role in the control of the output of phrenic motoneurons to the diaphragm. In the present study we report the anatomical localization of serotonin synaptic terminals on phrenic motoneurons in the cat using the double labelling technique of retrograde transport of HRP and EM immunocytochemistry. At the ultrastructural level both phrenic motoneurons and serotonin terminals were easily identifable. Serotonin immunoreactivity appeared as a diffuse reaction product throughout the cytoplasm of the synaptic terminal, while phrenic motoneurons retrogradely labelled with HRP contained distinctive spicules in the cytoplasm of the neuronal cell body and its dendrites. The serotonin immunoreactive terminals contained a mixture of small, clear vesicles and occasional larger, dense core vesicles. The terminals were in synaptic contact with both the cell body and dendrites of phrenic motoneurons. These anatomical findings are indicative of a direct serotonergic innervation of the phrenic motor nucleus

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379.8

GABA IMMUNOREACTIVE TERMINALS CONTACT PHRENIC MOTONEURONS. D.A. Vascik*, B.E. Maley, and J.R. Holtman Jr., Dept. of Anatomy and Neurobiology and Dept. of Pharmacology, Univ. of Kentucky Med. Ctr., Lexington, KY 40536.

GABA is a major inhibitory neurotransmitter which has a widespread distribution in the central nervous system. In the present report we describe its distribution in the phrenic motor nucleus of the cat both at the light and electron microscopic levels. Using a combination of immunocytochemistry and retrograde transport of HRP we were able to demonstrate that GABA immunoreactive terminals contact phrenic motor neurons that innervate the diaphragm.

GABA immunoreactivity is localized within puncta and small neurons throughout the the phrenic motor nucleus at the light microscopic level. At the ultrastructural level GABA immunoreactivity was located as diffuse reaction product within terminals containing small, clear vesicles that were in synpatic contact with neurons and all parts of their dendritic tree. GABA immunolabelled terminals contacted both HRP retrogradely labelled neurons and unlabelled neurons.

The presence of GABA immunoreactive terminals that contact phrenic motoneurons provides morphological evidence that GABA is involved in synaptic circuits that control the movement of the diaphragm. Supported by NIH grant NS23861 (B.E.M.) and NIH grants HL36050 and HL37146 (J.R.H.)

379.9

MICROINJECTION OF A GABA ANTAGONIST INTO THE POSTERIOR HYPOTHALAMUS BLOCKS THE RESPIRATORY RESPONSE TO BARORECEPTOR STIMULATION. G.H. Dillon*, C.A. Doyle* and T.G. Waldrop*.(SPON: M. Holzwarth). Dept. of Physiology, Univ. of Illinois, Urbana, IL 61801.

Prior work has shown that microinjections of a GABA antagonist into the posterior hypothalamus attenuate baroreflex bradycardia. The purpose of the present study was to determine if a similar mechanism modulates the respiratory response to baroreceptor stimulation (baro. stim.) in anesthetized rats. Respiratory responses to baro. stim. (elicited by increases in arterial pressure induced by phenylephrine injections) were determined before and after unilateral microinjections (50-100 nl) into the posterior hypothalamus (PH). Breathing frequency (f) and respiratory drive (Demg) were derived from diaphragmatic EMG recordings. During control conditions, baro. stim. elicited a decrease in f (-9 breaths/min) and a 7% fall in Demg. Microinjections of a GABA antagonist (picrotoxin, 5 ng/nl) into the PH produced increases in respiratory activity and blocked both the fall in f and Demg produced by baro. stim. These effects were reversed by PH microinjections of a GABA agonist (muscimol, 5 ng/nl). Microinjections of a GABA synthesis inhibitor (3-mercaptopropionic acid) also blocked the frequency response to baro. stim. We conclude that a GABAergic mechanism in the PH modulates the respiratory responses to baroreceptor stimulation. (Supported by NIH HL38726).

379.10

BENZODIAZEPINES POTENTIATE POSTSYNAPTIC INHIBITIONS IN BULBAR RESPIRATORY NEURONS OF CATS. R. Takeda and A. Haji*, Dept. of Pharmacol., Fac. of Med., Toyama Med. & Pharmaceut. Univ., Toyama 930-01, Japan.

We have studied the effect of benzodiazepines on the inhibitory postsynaptic potentials (IPSPs) and on the hyperpolarizing response to iontophoresed γ-aminobutyric acid (GABA) in bulbar respiratory neurons obtained from decerebrate, paralyzed cats. Diazepam (0.05-0.1 mg/kg i.v.) hyperpolarized the membrane in all inspiratory and postinspiratory neurons penetrated in the ventral respiratory group. Waves of IPSPs occurring during "inactive" phase of the respiratory cycle substantially increased and the input resistances decreased at that phase of each neuron. The effect during "active" phase include hyperpolarization associated with reduction of the input resistance, a decrease in the firing rate and shortening of the burst duration. Diazepam had no effect on the excitatory postsynaptic potentials induced either by the vagal or the spinal cord stimulation, whereas it increased the stimulus-induced IPSPs. Iontophoresis of flurazepam enhanced hyperpolarization induced by iontophoresed GABA. These results document that benzodiazepines potentiate specifically the phasic and tonic postsynaptic inhibitions in bulbar respiratory neurons, at least part of which are mediated by GABA.

379.11

SYSTEMIC ADMINISTRATION OF THE N-METHYL-D-ASPARTATE ACID (NMDA) RECEPTOR ANTAGONIST, MK-801 PRODUCES PRONOUNCED CHANGES IN CARDIORESPIRATORY FUNCTION. T.P.Abrahams*, R.A. Gillis*, P.Hamosh*, and A.M.Taveira da Silva*. (SPONS: Y. Tizabi). Departments of Pharmacology, Physiology and Medicine, Georgetown University Medical School, Washington D.C. 20007.

Because of the potential role of CNS excitatory amino acids in the control of cardiorespiratory function, we evaluated the effects of MK-801 on several indices of cardiorespiratory activity in chloralose-anesthetized cats. I.V. doses of 0.03, 0.1, 0.3 and 1mg/kg produced decreases in respiratory minute volume (VE) of 45±19, 145±25, 285±55 and 325±98 ml/min, respectively. The decrease in VE was due to both decreases in tidal volume (VT) and respiratory rate (f). The decrease in f was due to prolongation of inspiratory duration (Ti) as MK-801 (0.03, 0.1, 0.3 and 1mg/kg) increased Ti by 0.5±0.1, 0.8±0.1, 1.8±0.3 and 1.6±0.4 seconds respectively. The higher doses of MK-801 caused apneustic breathing and apnea. Biphasic changes in arterial pressure (BP) occurred that were dose-related. An initial increase (within 1 min) followed by a later decrease in BP (after 5 min of drug administration) were observed. No significant changes in heart rate were noted. These results indicate that NMDA receptor blockade with MK-801 causes respiratory depression and significant changes in BP (supported by MH42322).

379.12

ANTAGONISM OF THE RESPIRATORY EFFECTS OF SOMAN BY CLONIDINE AT THE LEVEL OF THE VENTRAL-LATERAL MEDULLA IN THE CAT. D.P. Walton*, R.A. Gillis*, P. Hamosh* and K.L. Dretchen (SPON: A. Raines). Depts. Pharmacol. & Physiol., Georgetown Univ. Med. Ctr., Washington, DC 20007.

It has been reported that systemic administration of clonidine will protect rodents against the toxic effects of soman (GD) (Buccofusco, 1986). Since GD exerts respiratory effects at the intermediate area on the ventral surface of the medulla (VSM) (Gillis et al., 1987), we determined whether GD-induced respiratory changes could be counteracted by prior treatment with clonidine applied at the VSM. GD (0.04 ug/side) applied bilaterally to the VSM of the chloralose-anesthetized cat decreased respiratory rate (f) by 9 + 2 breaths/min (from 15 + 1 breaths/min) and increased tidal volume (V_T) by 53 + 15 ml (from 32 + 2 ml); respiratory minute volume remained unchanged. Clonidine (1 ug/side) applied to the VSM 5 min prior to GD counteracted both the GD-induced decrease in f and the increase in V_T. GD (0.4 ug/side) produced apnea in 5 of 9 animals tested due to a decrease in f. Animals pretreated with clonidine did not exhibit apnea (0/4) when GD (0.4 ug/side) was applied. These data suggest that the protective effect of clonidine against GD toxicity may occur because of an interaction of the 2 drugs at the VSM. (Supported by the US Army Med. & Development Command, Contract #DAMD17-86-6034).

379.13

ROSTRAL VENTROLATERAL MEDULLA M_1 MUSCARINIC RECEPTOR INVOLVEMENT IN CENTRAL VENTILATORY CHEMOSENSITIVITY. E. NATTIE*, J. WOOD*, A. MEGA*, AND W. GORITSKI*. Department of Physiology, Dartmouth Medical School, Hanover, NH 03756. (SPON. F. McCann).

Muscarinic antagonists atropine (4.4 mM), the M_1 receptor antagonist pirenzepine (10 mM), and the M_2 antagonist AF-DX 116 (10 mM) were applied by cotton pledgets to the rostral ventrolateral medulla ventilatory chemosensitive area in separate anesthetized, paralyzed, vagotomized, glomectomized, and servo-ventilated cats with integrated phrenic nerve activity used as respiratory center output. Atropine or pirenzepine significantly decreased the CO_2 response slope 48.3% +/- 6.2 and 40.7% +/- 6.0 respectively and significantly decreased the maximum response value 26.3% +/- 8.1 and 19.2% +/- 3.2 respectively without significant effects on blood pressure. AF-DX 116 had no significant effect on phrenic output or blood pressure. Atropine or pirenzepine had no significant effect on the phrenic response to carotid sinus nerve stimulation. These results suggest the involvement of muscarinic cholinergic receptors of the M_1 subtype in the central CO_2 chemoreceptor process accessible to surface application of agents at the rostral ventrolateral medulla. (Supported by NIH HL 28066. Pirenzepine and AF-DX 116 supplied generously by Boehringer Ingelheim, Inc.)

379.14

CARDIORESPIRATORY RESPONSES FOLLOWING MICROINJECTIONS OF NECA INTO THE NTS OF DECEREBRATE RATS. M. El-Ridi*, C. Janusz*, M. Parizon*, E. Schoener* and R. Barraco. Department of Physiology, Wayne State University School of Medicine, Detroit, MI 48201.

Numerous studies have shown that adenosine and its analogs modulate neurotransmitter function in the brain. The Nucleus Tractus Solitarius (NTS) is a major relay nucleus for afferent input from cardiopulmonary receptors. Recent studies in our laboratory have shown that microinjections of adenosine and related drugs into the NTS produce potent cardiorespiratory responses and these responses exhibit a topographical pattern that depends upon which subnucleus is affected. The aim of the present study was to examine adenosinergic modulation of NTS function in decerebrate rats. A limited occipital craniotomy was conducted to expose the brainstem in the region of the obex for microinjections (25-50 nl) into the NTS. In some animals, a mid-collicular decerebration was made and the decerebration was confirmed via a stimulating electrode in the paraventricular nucleus of the hypothalamus. The results show that microinjections of adenosinergic drugs into the ventrolateral subnucleus of intact rats markedly depresses respiratory frequency and elicits pressor and depressor responses whereas only respiratory depression is seen in the decerebrate animal. (Supported by NSF (R1186-04084), NIH (RR-08167-10) and Am. Heart Assoc. of Mich.).

379.15

CORRELATION BETWEEN MUSCLE-FIBER TYPE AND GLYCOGEN CONTENT IN DIAPHRAGM. T.E. Dick, G.S. Supinski* and A.L. Freshler*, Depts. of Med. and of Physiol. and Biophys., Case Western Reserve Univ., Cleveland, OH, 44106.

We identified the fiber types recruited during resting ventilation in awake and anesthetized cats by correlating fiber type with glycogen content. Muscle fibers (>150/sample) were typed histochemically (myosin ATPase and NADH stains) and their glycogen content was assessed qualitatively as rich, partially depleted or poor (periodic acid-Schiff (PAS) stain). Glycogen content of a whole sample was determined quantitatively with anthrone reagent. For all animals (n=10), (Mean±SD) 40±5% of the muscle fibers were characterized as Type I (S), 19±4% as fatigue-resistant (FR) and 41±7% fatiguable (FF) Type II muscle fibers. In diaphragmatic samples (n=4) taken immediately after inducing anesthesia, no Type II and 18±13% Type I muscle fibers were glycogen poor; 60±15% S, 16±19% FR, and 10±9% FF muscle fibers were partially depleted. Glycogen was repleted by hyperventilating animals and infusing glucose (24% increase in whole muscle glycogen content after 8h, $P<0.05$). Then animals were spontaneously breathing at normocapnic levels for 1.5 h. Glycogen poor fibers were evident in both Type I and II fibers. Qualitatively, 78±18% S, 32±33% FR, and 28±20% FF fibers were depleted at least partially of glycogen. In conclusion, nearly all Type I and some Type II fibers are recruited in resting breathing. Support: HL 25830, Parker B. Francis Found., Amer. Lung Assoc. of Northern Ohio.

EXCITATORY AMINO ACIDS VII

380.1

NMDA RECEPTOR MEDIATES THE INITIAL SYNAPTIC RESPONSE IN SPINAL MOTONEURONS OF RAT EMBRYOS. L. Ziskind-Conhaim. Dept. Physiol., Univ. of Wisconsin, Madison, WI 53706.

Formation of sensory motoneuron contacts was studied in isolated spinal cord of rat embryos at 15-21 days of gestation using intracellular recording and HRP labeling (Ziskind-Conhaim, L., Dev. Biol., 128:1988). At Days 15-16, when most afferents terminated at the intermediate gray matter, dorsal root stimulation generated polysynaptic potentials. By Day 17, mono- and polysynaptic potentials were recorded in 60% of motoneurons, and HRP labeled afferents made synaptic contacts with motoneurons.

In the vertebrate central nervous system, at least 2 receptor subtypes (NMDA and kainate/quisqualate receptors) mediate the excitatory response to L-glutamate. At the onset of sensory innervation, the amplitide of polysynaptic potentials increased with depolarizing currents injected intracellularly and with removal of extracellular Mg^{2+}, a voltage-dependent blocker of the NMDA receptor. The prolonged potentiation induced by the removal of Mg^{2+} was blocked by 2-APV, a specific NMDA antagonist. These manipulations did not change the amplitude of the monosynaptic responses. These results suggest that NMDA receptor mediates the initial polysynaptic responses in the motoneurons of rat embryos. Conductance changes indicate that during embryonic development motoneuron sensitivity to NMDA and kainate was higher than to L-glutamate. Supported by NIH grant NS 23808 and by Spinal Cord Research Foundation grant NBR-489-5.

380.2

ROLE OF EXCITATORY AMINO ACIDS (EAAS) IN TRANSMISSION OF RESPIRATORY DRIVE TO PHRENIC MOTONEURONS I: KAINATE AND QUISQUALATE RECEPTORS. G. Liu, J.C. Smith, & J.L. Feldman, Systems Neurobiology Laboratory, Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

We previously demonstrated that excitatory amino acids are likely to play an important role in bulbospinal transmission of respiratory drive (*Fed. Proc. 45*:519, 1986). We report the effects of drugs affecting kainate and quisqualate receptor subtypes on phrenic motoneuronal resting membrane potential, respiratory drive potential and membrane conductance in the *in vitro* neonatal rat brainstem-spinal cord preparation (*J. Neurosci. Meth.21*:321, 1987).

To test the effects of blocking EAA receptors on transmission of respiratory drive, 2 mM Kynurenic acid (KYN), an antagonist acting on all three subtypes of EAA receptors, was applied to the solution bathing the spinal cord only. KYN reversibly abolished action potentials and significantly reduced (~75%) the amplitude of respiratory driving potential. Conductance measurements, using short current pulses, indicated that KYN specifically decreased transmitter mediated channel opening. Given the limited involvement of NMDA receptors in this phenomena (Feldman et al, this volume), these results suggested that non-NMDA receptors plays an important role in neurotransmission of respiratory drive. Thus, we tested the effects of a potent non-NMDA receptor antagonist, 6-cyano-7-nitroquinozaline-2,3-dione (CNQX). Bath application of 20 µM CNQX depressed respiratory drive potential and decreased receptor-mediated conductance changes, resulting in abolition of inspiratory-driven action potentials. Following wash out, phrenic motoneuronal discharge completely recovered. These results suggest that non-NMDA receptors are important in the synaptic transmission of bulbospinal respiratory drive to phrenic motoneurons. This work was supported by NIH Grant NS 24742. J.C.S. is a Parker B. Francis Foundation Fellow.

380.3

ROLE OF EXCITATORY AMINO ACIDS IN TRANSMISSION OF RESPIRATORY DRIVE TO PHRENIC MOTONEURONS II: N-METHYL-D-ASPARTIC ACID (NMDA) AND 2-AMINO-4-PHOSPHONOBUTYRIC ACID-SENSITIVE (AP4) RECEPTORS. J.L. Feldman, G. Liu, & J.C. Smith, Systems Neurobiology Laboratory, Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

Excitatory amino acids are likely to play a important role in bulbospinal transmission of respiratory drive (*Fed. Proc. 45*:519, 1986). Mechanisms of actions of drugs affecting NMDA and AP4 sensitive receptors were studied by intracellular recordings from phrenic motoneurons in the *in vitro* neonatal rat brainstem-spinal cord.

Addition of 200 µM 2-amino-5-phosphonovaleric acid (AP5), a specific NMDA antagonist, to the bathing medium surrounding the spinal cord, produced a modest decrease in respiratory drive potential. Increasing AP5 concentrations (< 1 mM) did not abolish phrenic motoneuron firing. Given the relatively high concentrations of AP5, the effects may be due to non-NMDA receptors. AP4 at low concentrations blocked action potentials and depressed driving potentials. Addition of 50-100 µM DL-AP4 to the spinal cord bathing medium completely blocked phrenic motoneuron activity within 1-2 min. The respiratory drive potential decreased gradually, with 90% reduction within ~10 min. Membrane conductance changes during this period suggest that DL-AP4 blocked neurotransmitter-evoked channel opening. Following replacement with control bathing solutions, respiratory driving potential and phrenic motoneuron discharge recovered gradually. Non-specific effect on membrane properties were tested by comparing neuronal rheobase before-during- after drug application; in all cases, rheobase was unaffected. The present results further demonstrate that although excitatory amino acids may transmit descending respiratory drive, NMDA receptors have only a limited involvement. AP-4 sensitive receptors, which may be pre- or post-synaptic, can powerfully affect the transmission of respiratory drive. Supported by NIH Grant NS 24742. J.C.S. is a Parker B. Francis Foundation Fellow.

380.4

THE NMDA RECEPTOR: CENTRAL ROLE IN MEDIATING PAIN INHIBITION IN RAT PERIAQUEDUCTAL GRAY. Y. F. Jacquet. Behavioral Neuropharmacology Lab., Nathan Kline Institute, Orangeburg, NY 10962.

The role of the periaqueductal gray (PAG) in the mediation of opiate analgesia is well established (Jacquet & Lajtha, Science, 1974). An injection of morphine in the PAG resulted in a naloxone-reversible analgesia, indicating an opiate receptor mediated action. The present study investigated what role, if any, the excitatory amino acids (EAA) may also play in pain inhibition at this CNS site. An injection of the EAA analogue, N-methyl-D-aspartate (NMDA)(10 nmol) in the rat PAG resulted in potent analgesia in 2 analgesia tests (Tail Flick & Noxious Pinch). A prior injection of the NMDA antagonist, (-)-2-amino-7-phosphonoheptanoate (D-AP7)(3 nmol) antagonized this action, indicating a receptor-mediated action. D-AP7 also completely blocked morphine analgesia. NMDA (10 nmol) given with morphine (25 nmol) potentiated morphine analgesia, while the opiate antagonist, naloxone (5 nmol), only partially reversed this analgesic action. These results are consistent with the view that opiate-mediated analgesia in the PAG may be due to a disinhibitory action on an excitatory descending pain inhibitory pathway (Basbaum & Fields, Ann Rev Neurosci., 1984). The present findings delineate for the first time a functional role for the NMDA receptor in the control of pain in the mammalian central nervous system.

380.5

ALTERATION OF EXTRACELLULAR GLUTAMATE AND GLUTAMINE LEVELS IN THE THALAMIC VENTROPOSTERIOR MEDIAL NUCLEUS (VPM) DURING VIBRISSAL STIMULATION AND FOLLOWING ABLATION OF THE SOMATOSENSORY CORTEX. D.T. Ross, Department of Clinical Neurosciences, Brown University and Department of Neurosurgery, Rhode Island Hospital, Providence, RI 02902.

An excitatory amino acid has been implicated as the neurotransmitter at lemniscal synapses in the thalamic ventrobasal complex on the basis of immunohistochemical, physiological, and pharmacological studies. In order to measure the release of excitatory amino acids during physiological stimulation *in vivo* microdialysis was performed on urethane (1.5 g/ Kg) anesthetized rats.

A microdialysis probe with a 2 mm long tip was stereotactically implanted in the VPM at a 45° angle along the long axis of the nucleus. Diaylsate samples were collected by infusing Ringer's solution through the probes at a rate of 2 µl /min for 30 minutes. Baseline samples were taken during normal spontaneous activity in a still room before and after stimulation. Continuous stimulation of the vibrissae for 30 minutes was accomplished by directing the air flow from a small fan onto the rat's face. After several episodes of stimulation, the SI cortex was ablated by subpial aspiration and dialysate samples were collected at 30 minuute intervals. Dialysate samples were diluted 1:1 with phosphate buffer, filtered, and 100 µl of the sample loaded onto a Beckman 7300 amino acid analyser. The chromatograms produced were examined for alterations in extracellular levels of glutamate, aspartate and glutamine.

Vibrissal stimulation produced a 13.3% increase in the measured level of glutamate in the VPM and a 43.0% increase in the level of glutamine. Baseline levels of aspartate in the VPM were very low and vibrissal stimulation did not produce a detectable increase. The extracellular concentration of glutamate and glutamine collected during ablation of the somatosensory cortex and for the subsequent 29 minutes were significantly lower than baseline levels, 65.3% and 59.7%, respectively. These results reflect the profound decrease in spontaneous activity recorded in the VPM immediately after cortical ablation. Studies in progress are examining alterations in extracellular amino acids in the VPM during cortical stimulation and at longer times following cortical ablation.

380.6

ROLE OF EXCITATORY AMINO ACIDS AND ADENOSINE IN CEREBELLAR CLIMBING FIBERS TRANSMISSION. K.Q.Do*, F.Vollenweider* and M.Cuénod (SPON: M. Schlumpf). Brain Research Institute, Univ. of Zürich, Zürich, Switzerland.

K^+-induced, Ca^{2+}-dependent in vitro release of endogenous aspartate (Asp), glutamate (Glu) and homocysteate (HCA) has been investigated in control and climbing fibers (cf) deprived rat cerebella. Degeneration of the inferior olive was induced by 3-acetylpyridine (3-AP). HCA release was abolished after 3-AP treatment. In hemisphere but not in vermis of 3-AP treated rats, the depolarisation induced release of Asp was decreased by 49% (from 43 to 27 pmole·mg protein^{-1}·min^{-1}) without changes in resting efflux. The decrease of 14% in K^+-induced release of Glu was mostly due to an increase in Glu resting efflux (from 26 to 43 pmole·mg protein^{-1}· min^{-1}). Moreover a Ca^{2+}-dependent K^+-induced release of adenosine (Ado) was observed: Ado efflux was increased from 8.2 (resting conditions) to 21.9 and 29.1 pmole·mg protein^{-1}· min^{-1} (during and after stimulation). In 3-AP treated rats, both the resting (-32%) and stimulated (-62%) efflux were decreased. Furthermore 50 nM of 1,3-dipropyl-8-cyclopentyl-xanthine, a selective antagonist of Ado at A1 sites increased both the basal and stimulated efflux of Glu in cerebellar slices. It is suggested that cf are using HCA and Asp as transmitters. Combined with work of others on Ado, our data are consistent with the hypothesis that cf activity induces release of Ado, which in turn depresses presynaptically the release of the parallel fiber transmitter, presumably Glu.

380.7

FAST EPSP'S EVOKED IN THE GOLDFISH MAUTHNER CELL BY SENSORY AFFERENTS ARE DUE TO NMDA RECEPTOR ACTIVATION. L. R. Wolszon* and D. S. Faber (SPON: J. Whitney). Dept. of Physiology, S.U.N.Y. at Buffalo, Buffalo, NY, 14214.

Saccular afferents terminate as mixed (electrotonic and chemical) synapses on the Mauthner Cell lateral dendrite. EPSP's produced by their impulses have time constants of decay in the range of 1-2 ms. To determine whether the transmitter at these synapses is an excitatory amino acid, we pressure-injected the glutamate antagonists γ-D-glutamyl glycine (γ-DGG) and 2-amino-4-phosphono valerate (APV) onto the lateral dendrite while recording, from that dendrite, EPSP's evoked by stimulation of the saccular nerve. The effects of these compounds on EPSP's arising from both single- and paired-pulse stimulation were examined. Neither drug altered resting membrane potential or antidromic spike height. γ-DGG completely abolished both the unconditioned and the facilitated EPSP's, whereas APV reduced each by at least 80-90%, indicating that the receptors at these synapses are predominantly of the NMDA subtype. For both compounds, the second, facilitated, EPSP was more sensitive to the antagonist than was the first.

We conclude that this fast EPSP is primarily due to the activation of NMDA receptors and that the paired-pulse facilitation of this EPSP seen with stimulation of a population of afferents may involve a postsynaptic mechanism, in addition to the presynaptic one described previously for single fibers (Lin, J.W and Faber, D.S (1988), J. Neurosci., 8 (4): 1313-1325). (Supported by NIH #NS 21848 and 15335)

380.8

A–DELTA AND C AFFERENT FIBER–EVOKED SYNAPTIC RESPONSES OF RAT SUBSTANTIA GELATINOSA NEURONS IN VITRO. M. Yoshimura* and T.M. Jessell*. Center for Neurobiology & Howard Hughes Medical Institute, Columbia University, New York, NY 10032.

Primary afferent fiber–evoked synaptic responses of substantia gelatinosa (s.g.) neurons have been analyzed by intracellular recording in a transverse slice preparation of adult rat spinal cord that retains an attached dorsal root.

Neurons in s.g. exhibited A–delta and/or C fiber mediated monosynaptic fast epsps in response to dorsal root stimulation. C and A–delta fiber–mediated epsps had similar time courses and amplitudes. To study C fiber input selectively we have used low concentrations (0.05 µM) of TTX that preferentially block the activation of A–delta fibers without substantially affecting C fiber evoked epsps. Higher concentrations of TTX (0.5 µM) blocked C fiber responses.

Both A–delta and C fiber evoked epsps were increased in amplitude with membrane hyperpolarization and decreased with membrane depolarization and reversed in polarity at membrane potentials between −10 and 0 mV. In the presence of TTX (0.5 µM), L-glutamate (0.5 – 5 mM) produced a membrane depolarization in 40–50% of s.g. neurons. The l–glutamate–induced depolarization reversed in polarity at the same membrane potential as that of afferent–evoked epsps. The amino acid antagonist kynurenate (1 mM) depressed the amplitude of A–delta and C fiber mediated epsps and l–glutamate response in some but not all s.g. neurons.

These results indicate that some but perhaps not all A–delta and C afferent fibers release amino acids as fast excitatory transmitters at afferent synapses with s.g. neurons.

380.9

SYNAPTIC PATHWAYS IN RAT PIRIFORM CORTEX. D.O. Carpenter and N.H. Hori*. School of Public Health, NYS Dept. of Health, Albany, NY 12237

We have characterized several synaptic pathways onto pyramidal neurons which can be selectively activated in rat piriform cortex slices. The pathway activated by stimulation of the lateral olfactory tract (LOT) is blocked by amino-phosphonobutyric (APB) and kynurenic acids, but not other excitatory amino acid antagonists. This pathway shows paired pulse (PPP) and long term potentiation (LTP), and afferents terminate on distal apical dendrites. Stimulation deep in the slice, which activates association fibers, elicits two distinct excitatory responses on pyramidal neurons. Both components are blocked by kynurenic acid but not APB or other amino acid antagonists. Neither component shows LTP, and only the faster shows PPP. Both components reverse deeper in the slice than the LOT inputs, the slower in the proximal apical dendrites while the faster appears to terminate on basal dendrites. GABAergic inhibition can be elicited by deep low threshold stimulation, and terminates near the cell bodies. Acetylcholine receptors are located exclusively on basal dendrites, and close voltage dependent M channels.

380.10

INITIATION OF SWALLOWING BY LARYNGEAL AFFERENTS: EXCITATORY AMINO ACIDS AS TRANSMITTER CANDIDATES. A. Jean*, N. Cherkaoui*, N. Schaffar*, J.P. Kessler* and D. Catalin*. (SPON: European Neuroscience Association) Lab. de Neurobiologie fonctionnelle. CNRS UA 205. Faculté St Jérôme. 13397 Marseille Cedex 13. France.

Swallowing is a motor activity generated by a central pattern generator including leading neurons located in the nucleus tractus solitarius (NTS). The sequential motor pattern characteristic of swallowing, linked with the inhibition of respiration, is routinely elicited in anesthetized rats by stimulation of laryngeal vagal afferents. The same motor pattern was also selectively generated by glutamate or excitatory amino acids (EAA) agonists (quisqualate, N-methyl-D-aspartate) applied into the swallowing region of the NTS (pressure injections: 5 nl, 0.05-0.5 nmol). Injections of a depolarizing agent (K^+, 0.06-2 M) were without effect. Moreover swallowing initiated by stimulation of laryngeal afferents was blocked by microinjections, into the active sites, of EAA antagonists (glutamate diethylester, γ-glutamylamino-methyl sulfonate, 2-amino-5-phosphonovalerate; 0.5-5 nmol). These results suggest that EAA might be transmitters involved in the initiation of swallowing by laryngeal afferents. Retrograde labeling of nodose cell bodies after injection of D-^{3}H-aspartate (100 µCi) in the swallowing region of the NTS supports the hypothesis that certain vagal afferent fibers use EAA as transmitters.

380.11

EVALUATION OF THE PHENCYCLIDINE-LIKE DISCRIMINATIVE STIMULUS EFFECTS OF THE PROPOSED NMDA ANTAGONIST NPC 12626. R.L. Balster*, J. Willetts and J.W. Ferkany (SPON: D.R. Compton). Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298 and NOVA Pharmaceuticals, Baltimore, MD.

The effects of the proposed competitive N-methyl-D-aspartate (NMDA) antagonist NPC 12626 were compared to those of 3-((+/-)-2-carboxy-piperazin-4-yl)propyl-1-phosphonic acid (CPP) and pentobarbital (PB) in rats trained to discriminate 1.25 mg/kg i.p. phencyclidine (PCP) from saline. Both CPP (3-30 mg/kg i.p.) and NPC 12626 (10-100 mg/kg i.p.) dose-dependently reduced response rates but failed to completely substitute for PCP. Similar effects were observed with PB. For all three drugs, PCP-lever responding was usually associated with response rate decreases, and response rate decreases often occurred without evidence of generalization. These results suggest that there is not a complete overlap in the discriminative stimulus effects of PCP and NPC 12626, CPP and PB. Furthermore, behavioral effects other than PCP-like effects are produced by these drugs, and the effects of the competitive NMDA antagonists are no more PCP-like than are the effects of PB.

(Partially supported by NIDA Grant DA-01442)

380.12

PENTOBARBITAL-LIKE DISCRIMINATIVE STIMULUS EFFECTS OF COMPETITIVE AND NON-COMPETITIVE NMDA ANTAGONISTS. J. Willetts and R.L. Balster*. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298.

Various competitive N-methyl-D-aspartate (NMDA) antagonists share certain behavioral effects with the non competitive NMDA antagonist phencyclidine (PCP), however, the selectivity of these drugs to produce only PCP-like effects has not been well-studied. We investigated whether the competitive NMDA antagonist 3-((+/-)-2-carboxy-piperazin-4-yl)propyl-1-phosphonic acid (CPP) also has effects similar to pentobarbital (PB).

The discriminative stimulus effects of CPP and the non-competitive NMDA antagonists MK-801 and PCP were assessed in 8 rats trained to discriminate 5 mg/kg i.p. PB from saline. We found that CPP completely generalized from PB at doses that did not affect response rates. However, neither PCP nor MK-801 completely substituted for PB; and, when present, PB-lever responding was accompanied by reduced response rates.

These results, along with our previous finding that CPP does not reliably substitute for PCP in PCP-trained rats, indicate that the behavioral effects of CPP may be more similar to PB than to PCP. (Supported by NIDA Grant DA-01442.)

380.13

6,7-Dinitroquinoxaline-2,3-dione (DNQX) and γ-aminomethylsulphonate (GAMS) selectively inhibit the hypermotility response produced by activation of quisqualic acid (QA) receptors in the nucleus accumbens (NA). R.C. Boldry*, P.E. Shreve*, N.J. Uretsky. (SPON: M.C. Gerald), The Ohio State University College of Pharmacy, 500 W. 12th Avenue, Columbus, Ohio 43210.

DNQX has been introduced as a selective competitive antagonist of non-NMDA receptors. The purpose of this study was to evaluate the ability of this compound to inhibit the hypermotility responses to excitatory amino acids (EAA) injected directly into the NA and to compare its effects with those of GAMS. Different doses of DNQX or GAMS were coinjected into the NA of rats with either kainic acid (KA), N-methyl-D-aspartic acid (NMDA), or α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA). Control rats received the EAAs alone. Low doses of DNQX or GAMS were found to selectively inhibit the response to AMPA, a QA receptor agonist, but to have no significant effect on the responses to NMDA and KA. Higher doses of DNQX or GAMS inhibited the responses to all three EAAs. Binding studies show that DNQX is a potent inhibitor of ^{3}H-AMPA binding while GAMS is ineffective except at higher concentrations. These studies show that both GAMS and DNQX can selectively inhibit the behavioral response to the intraaccumbens injection of AMPA. However, while direct antagonism of the QA receptor can account for the inhibitory effect of DNQX, the selective inhibitory effect of GAMS appears to be due to some other mechanism. Supported by NS 22582.

380.14

Kainic acid (KA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), and N-methyl-D-aspartic acid (NMDA) stimulate locomotor activity (LMA) after injection into the substantia innominata/lateral preoptic area (SI/LPO). P. E. Shreve* and N. J. Uretsky, The Ohio State University College of Pharmacy, 500 W. 12th Ave., Columbus, OH 43210.

The SI/LPO is a region of the ventral pallidum which appears to be a critical neural substrate for LMA initiated in the nucleus accumbens (NA) through a GABAergic projection from the NA. Recent evidence suggests that the SI/LPO also receives glutamatergic innervation. The purpose of these experiments was to determine the effects of excitatory amino acids (EAA) on LMA after direct injection into the SI/LPO. Various doses of KA, AMPA, and NMDA were injected into the SI/LPO and LMA was recorded. KA (15-60 ng), AMPA (0.1-1 μg), and NMDA (1-2.5 μg) produced dose dependent increases in LMA which was similar to that observed in the NA. γ-aminomethylsulphonate (GAMS) has been shown to be a selective quisqualic acid (QA) receptor antagonist in the NA. In the present study, GAMS (1 μg) produced a selective inhibition (82%) of AMPA (0.5 μg) while having no significant effect on KA (30 ng) or NMDA (2.5 μg). These results suggest that QA receptors mediate the LMA produced by AMPA and that activation of EAA receptors in the SI/LPO by glutamate may play a role in modulating goal-oriented behavior initiated in the NA. Supported by NS 22582.

380.15

CHANGES IN SPONTANEOUS LOCOMOTOR ACTIVITY AS A RESULT OF ADMINISTRATION OF KYNURENIC ACID IN THE RAT. Z. Dennison, K.-P. Ossenkopp and D.P. Cain. Dept. Psychology, Univ. of Western Ontario, London, Ontario, CANADA N6A 5C2.

Since excitatory amino acid antagonists have been shown to have anticonvulsant actions, it is important to examine the side effects produced by these compounds. Kynurenic acid has been shown to retard the rate of kindling and to act as an anticonvulsant in kindled animals. We examined the effects of intraventricular administration of kynurenic acid on locomotor behavior in rats. A Digiscan Activity Monitor was used to quantify a variety of behavioral measures. Male hooded rats were implanted with bilateral ventricular cannulae and amygdala electrodes. Four groups of animals (HI,MED,LOW,SAL) received 65, 39, 6.5 and 0 μg of kynurenic acid dissolved in saline, respectively. The drug was administered every other day, followed 40 minutes later by electrical stimulation in a standard kindling procedure. Movement data were collected on the first drug day and every third drug day thereafter. A repeated measures MANOVA was performed on horizontal activity and vertical (rearing) activity. The results indicated that HI and MED were significantly different from SAL on both measures ($p<.001$) but LOW was no different from SAL. Further analyses revealed different activity patterns within test sessions for various doses of kynurenic acid.

Supported by NSERC.

380.16

EFFECTS OF NMDA, L-GLUTAMIC ACID AND L-ASPARTIC ACID ON AUDITORY AND TACTILE STARTLE HABITUATION IN MICE. D. Helton*, J. Tizzano, J. Buelke-Sam, R.N. Tamura*, and P.D. Williams*. Toxicology Division, Lilly Research Laboratories, Greenfield, IN 46140.

The excitatory amino acids, N-methyl-D-aspartate (NMDA), l-glutamic acid (GLU) and l-aspartic acid (ASP) have been shown in our laboratory to exhibit proconvulsive activity in the mouse electoconvulsive seizure model. The present study was designed to evaluate the actions of these three compounds, at both proconvulsive (high) and non-proconvulsive (low) doses, on the startle reflex elicited by either auditory or tactile stimuli. Groups of 10 male CD-1 mice were randomly assigned to one of six drug conditions: 3.125 or 6.25 mg/kg NMDA, 500 or 1000 mg/kg GLU, 500 or 1000 mg/kg ASP. A total of 20 mice received saline injections. Intraperitoneal injections were administered in random order 15 min prior to startle testing in SDI chambers. Each 50-trial session was made up of alternating 5-trial blocks of auditory (120 dBA noise) and tactile (20 psi air puff) stimuli presented at 8-sec intervals. The low dose of NMDA did not alter auditory startle amplitudes compared to controls, but increased tactile startle during the first trial block; the high dose of NMDA decreased both auditory and tactile startle throughout the test session. The low dose of GLU resulted in a slight decrease in auditory startle during the first 3 trial blocks, but did not alter tactile startle responding; the high dose of GLU had no effect on startle elicited via either modality. Both doses of ASP produced comparable and consistent decreases (ca 40%) in auditory and tactile startle across trial blocks. These data indicate that non-proconvulsive doses of NMDA, GLU and ASP differentially altered startle responding. At proconvulsive doses NMDA and ASP, but not GLU, decreased both auditory and tactile startle amplitudes.

380.17

EFFECTS OF MK-801 ON MEMORY RETENTION IN THE RAT. D.F. Wozniak, J.W. Olney and L. Kettinger III*. Dept. of Psychiatry, Washington University Sch. of Med., St. Louis, MO. 63110.

Several experiments were conducted to study the effects of MK-801 on learning and memory processes in the rat. In one experiment, rats were trained on a task involving the reversal of a position habit. The protocol consisted of weekly, 3-day training periods. On day 1, rats (n = 38) were trained to go to one side of a T-maze to receive a food reward until they made 9 out of 10 consecutive responses to the reinforced side. On day 2, half of the rats were injected IP with saline and half with MK-801 (0.1 mg/kg), a potent antagonist of the N methyl-D-aspartate receptor-ionophore complex. Fifteen minutes later they were trained to go to the side of the T-maze opposite from the one in which they were reinforced on the previous day. Again, rats were required to make 9 out of 10 consecutive responses to the reinforced side. On day 3, they were given a 1-trial test to see if they would respond correctly, i.e., go to the side reinforced on day 2. The same experiment was repeated once a week for 3 consecutive weeks without crossing over experimental and control groups. The number of saline control rats which responded correctly on the 1-trial test was significantly greater than levels expected by chance for each of the three weekly experiments whereas MK-801 rats consistently failed to perform above chance levels. Performance deficits on day 3 could not be attributed to motor impairment or inebriation since these are acute types of drug effects which would not be expected to endure for 24 hrs; moreover, trials to criterion and choice latencies were not different between the 2 groups on day 2 when experimental rats learned the reversal under the acute influence of drug. The effects of MK-801 do not generalize to all aspects of memory since the drug did not impair working memory tested in a radial arm maze or in a reinforced spatial alternation task. Our experiments suggest that rats are capable of learning while under the acute influence of MK-801 (0.1 mg/kg) but may have difficulty recalling what was learned when tested 24 hrs later. Supported by Research Scientist Award MH 38894 (JWO), AG 05681 and MH 14677.

380.18

MK-801 PREVENTS COGNITIVE AND BEHAVIORAL DEFICITS PRODUCED BY N-MDA RECEPTOR OVERSTIMULATION IN THE RAT HIPPOCAMPUS. B.C. Rogers*, and H.A. Tilson. LMIN, Nat. Inst. Environ. Health Sciences, Research Triangle Park, NC 27709.

We report that functional and histological damage mediated by the intrahippocampal bilateral injections of N-methyl-D-aspartate (N-MDA) into the hippocampus can be attenuated by the administration of MK-801 (i.p.) 1 hr prior to treatment. First, MK-801 (10.0 mg/kg) was administered prior to increasing doses of N-MDA (5,10, and 20 ug/site). Next, two doses of MK-801 (1.0 and 0.1 mg/kg) were administered prior to one dose of N-MDA (10 ug/site). In both experiments, motor activity was measured 1, 2, and 3 wk after surgery. Also, animals receiving 10 ug/site N-MDA were trained for acquisition of a Morris water maze task 4 wk post-surgery. 10 mg/kg MK-801 completely blocked increases in motor activity produced by 10 and 20 ug/site N-MDA while both 1.0 and 0.1 mg/kg MK-801 abolished 10 ug/site N-MDA-induced hyperactivity. In addition, 10.0 and 1.0 mg/kg MK-801 completely attenuated water maze acquisition deficits produced by N-MDA. Finally, histological examination revealed 10.0 mg/kg MK-801 provided complete protection from damage produced by 5 and 10 ug/site N-MDA while 1.0 and 0.1 mg/kg site MK-801 also prevented cell loss to a lesser extent. These results suggest that MK-801 may be effective in attenuating memory loss associated with the overstimulation of N-MDA receptors. (B.C.R. supported by ES01726.

380.19

RESPIRATORY APNEUSIS FOLLOWING N-METHYL-D-ASPARTATE (NMDA) RECEPTOR BLOCKADE. A.S. Foutz*, G. Fortin*, J. Champagnat* and M. Denavit-Saubié, Biologie Fonctionnelle du Neurone, L.P.N. C.N.R.S. 91190 Gif-sur-Yvette (France).

We investigated in-vivo and in-vitro the functional role of NMDA receptors in the brainstem and the possible neuronal networks involved. Systemic administration of NMDA antagonists (MK-801 and phencyclidine 0.03-1 mg/kg ; ketamine 2.5-10 mg/kg ; AP7 120 mg/kg to decerebrated, vagotomized and paralyzed cats increased the duration of central inspiratory activity (Ti) recorded from the phrenic nerve without increasing the duration of expiration. Suppression of pulmonary vagal afferent input was required for obtaining such apneusis. Cats given MK-801 with intact vagus nerves were ventilated (100% O_2) with a pump driven by the discharge of the phrenic nerve. The omission of lung inflation increased Ti from 1-2 sec to up to 2 min. These results were confirmed in chronic cats given MK-801 before and after vagotomy.

Therefore : 1) NMDA-type glutamate receptors are involved in respiratory rhythmogenesis (inspiratory off-switch) ; 2) these receptors are activated by aminoacidergic neurons located within the brainstem. We have looked in-vitro for the possible neuronal basis of these in-vivo results. Coronal brainstem slices perfused with a magnesium-free medium provided evidence for a local network in the dorsal respiratory area involving dicarboxylic aminoacid release and NMDA receptor activation.

380.20

DEPOLARIZING SYNAPTIC EVENTS INFLUENCING CAT LUMBAR MOTONEURONS DURING RAPID EYE MOVEMENT EPISODES OF ACTIVE SLEEP ARE BLOCKED BY KYNURENIC ACID. P.J. Soja, F. Lopez*, F.R. Morales, and M.H. Chase *Depts. of Physiology and Anatomy, and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.*

The membrane potential activity of lumbar motoneurons during rapid eye movement (REM) periods of active sleep (AS) is characterized by glycinergic inhibitory processes, as evidenced by brief periods of strychnine-sensitive hyperpolarization (*Sleep Res. 14:* 4, 1985), as well as by complex patterns of paroxysmal depolarizing shifts that lead to full-size or partial amplitude action potentials (*Science, 221:* 1195-1198, 1983; *Neuroscience Lett., 34:* 177-182, 1982). The neurotransmitter(s) responsible for the state-dependent excitatory processes is not known. Accordingly, we examined the effects of excitatory amino acid antagonists on the phasic depolarizing events that occur during REM periods of AS in the chronic cat.

Juxtacellular microiontophoretic applications of the non-selective excitatory amino acid antagonist, kynurenic acid (KYN, 0.15M, pH8.0, -100 to -300nA), were performed during wakefulness (W), quiet sleep (QS) and the transition into and throughout AS. Intracellularly recorded compound mono- and polysynaptic EPSPs, evoked by low-intensity sciatic nerve stimulation during W and QS, were markedly suppressed or abolished by KYN; short latency IPSPs were not blocked, indicating that the drug was adequately released and specific for excitatory neurotransmission. During REM periods of AS, KYN suppressed the naturally occurring phasic depolarizing membrane shifts and action potentials. Membrane potential activity during REM periods following KYN was characterized only by marked recurrent hyperpolarizations. Microiontophoresis of the selective N-methyl-D-aspartate (NMDA) antagonist, 2-amino-5-phosphonovaleric acid (APV, 0.2M, pH8.0, -100 to -300nA), suppressed polysynaptic EPSPs evoked by sciatic nerve stimulation and motoneuronal depolarizations induced by juxtacellularly applied NMDA, but failed to block the naturally occurring phasic depolarizing membrane shifts during the REM periods of AS.

On the basis of the documented selectivity of KYN and APV in antagonizing the postsynaptic actions of NMDA versus non-NMDA-like excitatory amino acids, the present results suggest that the postsynaptic excitatory drives that impinge on lumbar motoneurons during the REM periods of AS are mediated primarily by a non-NMDA-like neurotransmitter. Supported by grants NS2346 and MH43362.

EXCITATORY AMINO ACIDS VIII

381.1

CGP 37849 / CGP 39551: POTENT AND SELECTIVE COMPETITIVE NMDA RECEPTOR ANTAGONISTS WITH ORAL ACTIVITY. G.E. Fagg, H-R. Olpe*, H. Bittiger*, M. Schmutz*, C. Angst*, D. Brundish*, H. Allgeier*, R. Heckendorn* & J.G. Dingwall*, Friedrich Miescher Institute, Central Research Labs., & Research & Development Dept., Pharmaceuticals Division, CIBA-GEIGY Ltd., 4002 Basel, Switzerland.

As part of a rational approach to the development of novel NMDA (N-methyl-D-aspartate) receptor antagonists, we investigated a series of unsaturated analogues of the known blocker, AP5. Compounds were selected on the basis of their activity at NMDA-sensitive L-^{3}H-glutamate binding sites in rat brain synaptic membranes and as antagonists of experimentally-induced seizures in mice. CGP 37849 (D,L-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid) inhibited L-^{3}H-glutamate and ^{3}H-CPP binding with Ki values of 220 and 35 nM, respectively (comparative values for DL-AP5 were 1.5 and 0.5 μM), and was weak or inactive in assays for 17 other receptor types (including quisqualate and kainate). Sub-micromolar concentrations blocked NMDA-, but not quisqualate- or kainate-evoked increases in pyramidal cell firing in the rat hippocampal slice *in vitro*. It is thus the most potent and selective competitive NMDA receptor antagonist described to date. In rodents, CGP 37849 and its ethyl ester CGP 39551 (which was weaker in assays *in vitro*) potently antagonized electroshock-induced seizures following oral administration (ED_{50} values 4-21 mg/kg)(for details of their anticonvulsant profiles, see M. Schmutz et al., this meeting).

CGP 37849 and CGP 39551 thus are new drugs with which to examine the roles of NMDA receptor-mediated events in brain function, and are potential candidates for the treatment of disorders such as epilepsy and ischaemic neurodegeneration.

381.2

MODULATION OF A10 DOPAMINE BY EXCITATORY AMINO ACIDS. P.W. Kalivas and P. Duffy*. Dept. of VCAPP, Wash. St. Univ., Pullman, WA 99164

Do excitatory amino acids activate dopamine (DA) neurons in the A10 region? Glutamic acid, aspartic acid, kainic acid or N-methyl-D-aspartate (NMDA) were injected into the A10 region of rats, changes in motor activity and DA metabolism measured. Glutamate and kainate produced a dose-related increase in horizontal photocell counts with a minimum effective dose (MED) of 100 and 1 nmoles, resp. The dose response curves for NMDA and aspartate were biphasic. An increase in activity was seen at high doses of NMDA and aspartate (1 and 100 nmoles), while a decrease occurred after lower doses (0.01 and 10 nmoles). When a behavioral stimulant dose of kainate and glutamate was injected into the A10 region, a significant increase in DA metabolism was produced in both the A10 region and nucleus accumbens, and when a behavioral depressant dose of NMDA was given, an increase in DA metabolism was seen only in the A10 region. In spite of the behavioral depressant effect of low doses of NMDA, it significantly potentiated the motor stimulant effect of kainate to an MED of 0.1 nmoles. Also, a behavioral depressant dose of NMDA potentiated the behavioral stimulant effect of DAGO, a mu opioid agonist. These data show that kainate receptor stimulation activates the DA neurons to increase both somatodendritic and terminal field DA neurotransmission. In contrast, activation of the NMDA receptor appears to enhance only somatodendritic DA neurotransmission.

381.3

LOSS OF [^{3}H]GLUTAMATE BINDING AFTER ORBITAL ENUCLEATION
J. McCulloch & D.T. Chalmers*, (SPON. G. Fink), Wellcome Surgical Institute, University of Glasgow, Glasgow G61 1QH.

Glutamate plays a major role within the rat visual system. Using a fully quantitative autoradiographic technique, we have examined the alterations in [^{3}H]-glutamate binding sites which accompany functional disturbances after lesioning of this sensory pathway.

Black-hooded Long Evans rats were unilaterally enucleated under 2% halothane anaesthesia and twenty-four hours later, cerebral glucose use was measured using the (^{14}C)-2-deoxyglucose technique. (^{14}C)-2-deoxyglucose-6-phosphate was eluted from those sections required for receptor autoradiography and these were incubated for 45 min. at 4°C with 200nM [^{3}H]-glutamate, non-specific binding being determined in the presence of 1mM unlabelled glutamate.

Orbital enucleation produced a significant reduction in glucose use in the visually deprived superior colliculus, lateral geniculate nucleus and visual cortex. These functional disturbances were accompanied by a significant reduction in [^{3}H]-glutamate binding in both superficial and deep layers of visual cortex.

This rapid response to altered neuronal activity suggests an important functional role for this site in cortical excitatory neurotransmission. This response will be discussed in relation to specific receptor subtypes and binding conditions.

381.4

KAINIC ACID INDUCES HEAT SHOCK PROTEIN IN SPECIFIC BRAIN REGIONS.
Kathleen R. Zahs*+, Manuel F. Gonzalez, Kinya Hisanaga*, Stephen M. Sagar, and Frank R. Sharp+, (SPON: S.F. Akana). Depts. of Physiology+ and Neurology, UCSF, and VA Medical Center, SF, CA 94121 USA.

Following transient ischemia or hyperthermia, specific proteins have been found to be induced in mammalian brains. The present experiments demonstrate that administration of an excitatory neurotoxin also induces such heat shock proteins, suggesting that these proteins may serve as a marker for injured neurons.

Three rats received subcutaneous injections of kainic acid (10 mg/kg) and were killed 3 days later. Heat shock protein was detected using a mouse monoclonal antibody directed against a 72-kD heat shock protein. Immunostaining was observed in hippocampal layers CA1, CA2, CA3, and the parafascicular nucleus of the thalamus. In addition, Golgi-like staining was seen in layers II / III, V, and lower layer VI of piriform and perirhinal cortex. Cerebellar Purkinje cells were lightly stained. Injections of kainate at a dose that fails to cause neuronal death (3 rats, 6 mg/kg) resulted in no detectable staining of these regions.

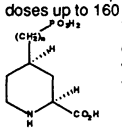
Local injections of kainate into hippocampus (0.4 microgram in 0.3 microliter) resulted in staining of ipsilateral hippocampal regions CA1, CA2, and CA3 and of the amygdala bilaterally.

381.5

EFFECTS OF A SERIES OF 4-(PHOSPHONOALKYL)PIPERIDINE-2-CARBOXYLIC ACIDS IN PIGEONS AND MICE. SELECTIVE ANTAGONISTS OF NEUROTRANSMISSION AT NMDA PREFERRING EXCITATORY AMINO ACID RECEPTORS.
P.L. ORNSTEIN*, J.D. LEANDER* AND J.W. CHAMBERS* (SPON: D. GOLDSTEIN). Lilly Research Labs, Lilly Corporate Center, Indianapolis, IN 46285.

We recently prepared a series of 4-phosphonoalkylpiperidine-2-carboxylic acids (**I**, n = 1; **II**, n = 2; **III**, n = 3; **IV**, n = 4) as antagonists of neurotransmission at N-methyl-D-aspartate (NMDA) preferring receptors. In order to delineate the extent of antagonist activity, we examined these compounds in a variety of assays: production of phencyclidine (PCP)-like catalepsy and antagonism of NMDA-induced suppression of responding in pigeons; protection from maximal electroshock (MES)-induced convulsions in mice; protection from NMDA- and 3-mercaptopropionic acid (3-MPA)-induced lethality in mice; impairment on the horizontal screen (mice).

Compounds **I** and **III** produced PCP-like catalepsy (both 2.5 mg/kg, i.m., slow onset and long duration of action) and antagonized NMDA-induced suppression of responding (both 0.16-0.32 mg/kg, i.m.) in pigeons, while **II** and **IV** were inactive (>40 mg/kg). In mice, **I** and **III** protected against MES-induced convulsions (10 and 40 mg/kg, i.p., respectively), 3-MPA-induced lethality (both 80 mg/kg, i.p.), and NMDA-induced lethality (10 and 40 mg/kg, i.p., respectively). At doses that afford protection in the above assays, the mice were impaired on the horizontal screen. Compounds **II** and **IV** (at doses up to 160 mg/kg, i.p.) were inactive in mice.



The pattern of activity observed for this series parallels that observed for the acyclic series of ω-phosphono-α-amino acids, where AP5 and AP7 possessed NMDA antagonist activity while AP6 and AP8 were inactive. Reduction of conformational mobility by incorporation of the piperidine ring led to enhanced potency relative to the acyclic analogues.

381.6

ALTERATIONS IN RAT BRAIN METABOLIC ACTIVITY BY VARIOUS NON-COMPETITIVE NMDA ANTAGONISTS.
H.D. Everist, J. Monn*, K. Rice* and A. Pert (SPON: L. Hsu). BPB, NIMH, and LC, NIDDK.

The purpose of this study was to compare the effects of three non-competitive NMDA antagonists on the metabolic activity of rat brain. Rats were injected systemically with either phencyclidine (PCP), (+) SKF 10,047 (-) SKF 10,047, MK-801 or saline. Thirty minutes later, all animals were injected with [^{14}C]-2-deoxyglucose (2-DG). Standard procedures of visualizing 2-DG were employed.

Significant increases in metabolic activity were found in the anterior and posterior cingulate cortex, anteroventral, ventromedial and posterior thalamic nuclei, dorsal and ventral hippocampus, and substantia nigra (ZR) following MK-801. PCP also enhanced metabolic activity in the anterior and posterior cingulate and the substantia nigra (ZR), as well as the caudate nucleus. Unlike MK-801, however, PCP decreased metabolic activity in the thalamic nuclei. In general, both enantiomers of SKF 10,047 reduced the metabolic activity of a variety of structures, including the hippocampus, the majority of thalamic nuclei and the substantia nigra. It is clear that these non-competitive NMDA antagonists have vastly different consequences on brain metabolic activity.

381.7

CENTRALLY ADMINISTERED QUISQUALIC ACID INCREASES SENSITIVITY TO DIETHYLBARBITURIC ACID IN LS AND SS MICE
C. C. Duncan and J. A. Ruth. University of Colorado School of Pharmacy, Box 297, Boulder, CO, 80309.

A gas-chromatographic assay for the determination of brain diethylbarbituric acid (DB) concentrations has been developed and used to study the role of glutamate neurotransmission in the modulation of the CNS depressant effects of this water soluble barbiturate in long-sleep (LS) and short-sleep (SS) mice. LS and SS mice are lines genetically selected for differential response to acute doses of ethanol as measured by duration of loss of righting response. We have previously demonstrated a differential involvement of NMDA-type glutamate receptors in this response. The current study was designed to extend the ethanol findings using other central depressants.

Mice were injected IP with 15 mg/kg DB. DB brain concentrations were determined at loss of righting response. LS and SS differed significantly in central sensitivity to DB (77.0±7.5 μg/g brain in LS and 120.0±4.0 μg/g brain in SS). Time course studies showed that these diffences were not due to differences in DB clearance rates. These data provide direct evidence for the differential sensitivity of LS and SS mice to the depressant effects of DB. The role of glutamate modulation of DB effects was studied by ICV administration of the NMDA antagonist, D-2-amino-5-phosphonovaleric acid (5-APV). 5-APV had no effect on brain sensitivity to DB in either mouse line. Quisqualic acid (QQA), the non-NMDA receptor agonist, caused a significant increase in central sensitivity to DB as reflected by a 45% decrease in brain DB concentration which induced loss of righting. These data support a role of glutamate neurotransmission in the modulation of the central-depressant effects of water soluble barbiturates.
Supported by USPHS grant AA-03527.

381.8

NMDA RECEPTORS ARE INVOLVED IN THE ACUTE RESPONSE TO ETHANOL IN LS AND SS MICE.
W. R. Wilson and J. A. Ruth, University of Colorado School of Pharmacy, Box 297, Boulder, CO, 80309.

LS and SS are lines of mice that have been genetically selected for their differential response to acute ethanol as measured by duration of loss of righting response. We have shown that the NMDA antagonist 2-amino-5-phosphonovalerate (APV) given by ventricular injection increased CNS sensitivity to ethanol 55% in LS and 45% in SS mice. NMDA had a small effect in LS mice but was without effect in SS mice. The current study was designed to further investigate the role of NMDA receptors in the response to ethanol. Halothane anesthetized mice were injected *icv* with 5μl of drug dissolved in physiological saline. Ten minutes later ethanol was administered *ig* (LS 7g/kg, SS 12g/kg). At loss of righting response blood samples and the brain were analyzed for ethanol content (BEC). Quinolinic acid (QA), an NMDA agonist, significantly and differentially decreased sensitivity of both LS and SS mice to ethanol as shown by increases of 40% (414mg%±15) and 30% (604mg%±22) respectively in BEC at loss of righting response. APV coinjected with QA blocked the effects of QA in a dose related fashion. $MgCl_2$ an antagonist of the NMDA receptor channel increased sensitivity to ethanol in both lines, as shown by a 15% (LS 254±18) and 20% (SS 392±27) decrease in BEC at loss of righting response. When QA was coinjected with $MgCl_2$ the effect of QA was decreased to saline control levels (LS 295mg% ±16; SS 401mg%±37). This study supports the hypothesis that NMDA receptors are involved in the acute response to ethanol in LS and SS mice and may be part of the biochemical profile that is involved in the differential response to ethanol in LS and SS mice.
This work was supported by USPHS grant AA-03527.

381.9

THYROTROPIN RELEASING HORMONE ENHANCES EXCITATORY SYNAPTIC TRANSMISSION IN VOLTAGE CLAMPED FROG MOTONEURONES. G. Lacey* and A. Nistri. Department of Pharmacology, St. Bartholomew's Hospital Medical College, University of London, London EC1M 6BQ, Great Britain.

Thyrotropin releasing hormone (TRH) facilitates motoneuronal reflex discharges in the spinal cord. Here we have examined the effect of TRH on the excitatory postsynaptic currents (EPSCs) of motoneurones.

Longitudinal slices of frog (Rana temporaria) spinal cord were superfused with Ringer solution containing 1 mM Mg^{2+} at 7°C. Single microelectrodes filled with 3M KCl and connected to an Axoclamp 2A were used to voltage clamp motoneurones (switching frequency 1.5 - 2.0 kHz).

Results were obtained from 12 motoneurones (resting potential 79 ± 1 mV). Polysynaptic current amplitudes were measured over a range of holding potentials (-80 to -30 mV) both before and after the addition of TRH (50 μM). No sustained inward or outward current was produced by TRH, but in some cases there was an increase in the frequency of spontaneous synaptic currents. In 8 of the cells tested, TRH caused a significant ($P < 0.02$) increase in the amplitude of the EPSCs over the entire range of holding potentials used. The reversal potential of the EPSC was estimated, by extrapolation of the I/V curve, to be approximately + 5 mV and was unchanged by TRH. The I/V curves were non-linear, particularly over the range -60 to -80 mV, suggesting a voltage dependence of the channels involved in the production of the EPSC, reminiscent of those channels activated by exogenous glutamate in these neurones.

Our previous work has shown that the membrane conductance of motoneurones and their sensitivity to exogenous glutamate are unchanged by TRH. Together, these results provide evidence for a presynaptic site of action of TRH. This work is supported by the Wellcome Trust.

381.10

SEX STEROIDS PRODUCE DIFFERENTIAL ELECTROPHYSIOLOGICAL EFFECTS ON EXCITATORY AMINO ACID AND GABA RECEPTOR SUBTYPES IN THE CEREBELLUM. Sheryl S. Smith, Dept. of Anatomy, Hahnemann U., Philadelphia, PA 19102-1192.

Ongoing studies in this laboratory have demonstrated that systemically administered sex steroids 17β estradiol (E_2) and progesterone (P) alter cerebellar Purkinje (Pnj) cell responses to the amino acids GABA and glutamate (Glu) in the adult female rat. In the present study, in an attempt to delineate the specific receptor subtypes responsible for the observed steroid effects, Pnj cell responses to iontophoretically applied (20s every 50s at 5-50 nA) Glu agonists quisqualate (Quis), kainate and NMDA, as well as to specfic NMDA antagonists such as APV, were tested before and after i.v. application of E_2 (100 ng/kg) or P (50 μg). In addition to a direct excitation, continuous application of NMDA can also produce marked increases (up to 400%) in Quis excitation (22/30 cells). Differential effects of P on $GABA_A$ vs. $GABA_B$ receptors were also examined using baclofen and bicuculline.

The Glu-enhancing actions of E2 appear to have two components: a fast effect (5-10 min) exerted at the Quis receptor and a later component involving NMDA receptor activation: E2 augmented Quis excitation by 100% (35/39 cells), an effect which could only be blocked by APV 20 min post-steroid. Although E2 effects on direct NMDA actions were inconsistent, this steroid produced a 40% increase in NMDA enhancement of Quis excitation. No effects on kainate excitation were noted. In contrast, P depressed excitatory responses of Pnj cells to both Quis (33/36 cells) and kainate (10/11 cells) by 30-70%. Surprisingly, P also potentiated the modulatory effects of NMDA on Quis responses. This latter action may moderate P effects on Glu physiology, as concurrent application of APV increased the magnitude of P suppression of Quis excitation by 10-20%, and prevented recovery to control levels of Quis excitation normally seen by 30 min post-P. P effects on the GABA receptor were found to be predominantly mediated at the $GABA_A$ subtype, as i.) No effects of P on Baclofen inhibition were observed and ii.) A dose of bicuculline resulting in a 40% reduction in GABA inhibition prevented P modulation of GABA responses. Furthermore, P modulation of Quis action was determined to be independent of the $GABA_A$ receptor, as bicuculline application did not prevent P suppression of Quis excitation. *(Supported by NS25809.)*

381.11

THE ROLE OF NMDA RECEPTOR MEDIATED CURRENTS IN THE MANIFESTATION OF LTP IN CORTICAL NEURONES. A. Artola and W. Singer. Max Planck Institute for Brain Research, D-6000 Frankfurt/M 71, F.R.G.

In pyramidal cells of rat visual cortex slices, reduction of GABAergic inhibition increases NMDA receptor mediated currents and facilitates long term potentiation (LTP) after tetanic stimulation (TET) of the white matter (Artola A. and Singer W., Nature 330:649). To test the hypothesis that NMDA currents are crucial for the induction and manifestation of LTP we bath applied bicuculline (BIC) at various concentrations (0.1 to 0.5 μM). The BIC effect was quantified by comparing the amplitude of a delayed PSP component (l-PSP), which contains an NMDA mediated potential to that of the early, non-NMDA dependent, PSP component (e-PSP, 8-11 ms poststimulus). At 0.1 to 0.2 μM, BIC neither modified the l/e-PSP ratio (33.6 ± 4.7%,S.D., n=8; control 35.5 ± 9.1%, n=11) nor did it facilitate LTP. At higher concentrations BIC increased the ratio and TET induced LTP. With small ratio increases (l/e = 58.9 ± 11.6%, n=8) only the l-PSP underwent LTP, with large ratio changes (91.3 ± 38.1%, n=11), both e- and l-PSP showed LTP, which amounted to 144.6 ± 22.0% and 127.0 ± 16.1% of control, respectively. APV reversed LTP only of the l-PSP. These results confirm that NMDA conductances are necessary for the induction of LTP and are antagonized by GABA. They indicate further that LTP increases first the NMDA dependent EPSP and, if strong enough, also the non-NMDA EPSP.

381.12

EFFECTS OF NEW KAINATE RECEPTOR ANTAGONISTS ON MUDPUPPY RETINAL NEURONS. E.A. COLEMAN* and R.F. MILLER. DEPT of OPHTHALMOLOGY, WASHINGTON UNIVERSITY, ST. LOUIS, MO 63110. (spon. A.I. Cohen)

We have analyzed the action of 6 new putative kainate (KA) receptor antagonists using whole cell recording techniques in the intact mudpuppy retina. These antagonists were bath applied and in some cases, tested against the excitatory amino acid receptor (EAAR) agonists. The quioxaline derivatives DNQX and CNQX (Honore, et al., 1987, Soc. Neurosci. Abstr. p.383) significantly reduced, if not completely abolished, the light evoked response of 80% of the horizontal and ganglion cells tested at a concentration of 25 μM. Antagonists such as kynurenate required concentrations as high as 3-5 mM to achieve similar effects. A related drug, 6,7-Dichloro-3-hydroxy-2-quinoxalinecarboxylic acid was also effective in reducing synaptic responses but at higher concentrations (100 μM). However, 3-Hydroxy-2-quinoxalinecarboxylic acid, at 100 μM, had virtually no effect on ganglion cell photic responses. Another group of EAAR antagonists were also tested: MLV-6976 and MLV-5860 (Masaki & Shinozaki, 1986, Br. J. Pharmac. 89, 219-228). These compounds also reduced the light evoked response of retinal ganglion cells but at a concentration of 1 mM.

CNQX and DNQX were also tested against exogenously applied EAAR agonists: kainate, N-methyl-D-aspartate (NMDA), and quisqualate (QQ). Both compounds, at 25 μM, blocked the depolarizing effects of KA (50 μM) and QQ (15 μM). CNQX blocked the depolarizing effects of NMDA (300-500 μM) in 40% of the cells tested. In the remaining cases, CNQX reduced the NMDA response to < 50% of the control level. DNQX, which was slightly more effective in reducing the synaptic responses, always blocked the depolarizing effects of NMDA. Though not the most selective of the EAAR antagonists we have tested, CNQX and DNQX are by far the most potent.

381.13

BARIUM REVEALS NMDA RECEPTOR-MEDIATED SYNAPTIC TRANSMISSION IN TURTLE HIPPOCAMPUS. Linda J. Larson-Prior and N.Traverse Slater, Dept. of Physiology, Northwestern University Medical School, 303 E. Chicago Avenue, Chicago, IL 60611 U.S.A.

NMDA receptor antagonists reduce convulsant-induced epileptiform discharges in turtle hippocampus (ventromedial cortex), but in the absence of convulsant drugs afferent stimulation of VMC pyramidal neurons evokes a biphasic IPSP which masks underlying excitatory transmission [1]. Direct evidence for NMDA receptor-mediated transmission in this structure has been demonstrated by the bath application of 500 μM $BaCl_2$ or penetration of cells with cesium-filled electrodes, both of which block the late K^+-mediated IPSP and reveal a late, slow EPSP (Fig. 1). This EPSP was reversibly blocked by the NMDA receptor antagonists DL-AP5 (50-100 μM) and ketamine (250 μM).

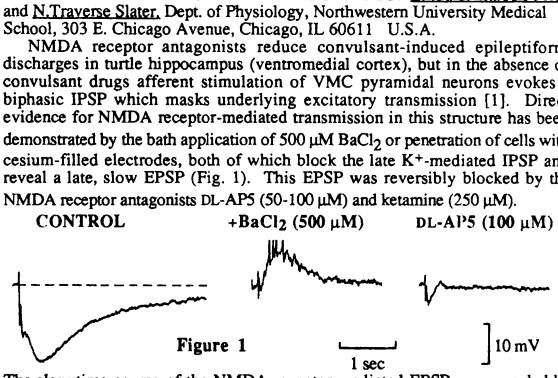


Figure 1

The slow time-course of the NMDA receptor-mediated EPSP was revealed by subtraction of responses before and after the addition of DL-AP5 or ketamine. I-V plots of this potential did *not* display the expected voltage-dependent rectification in the presence of external magnesium. Voltage-clamp of neurons in barium or impaled with cesium-filled electrodes revealed a slow inward current which was reversibly blocked by DL-AP5. These results demonstrate the existence of NMDA receptor-mediated excitatory transmission in turtle hippocampus that likely contributes to the maintenance of convulsant-induced epileptiform discharges.

[1] L.J. Larson-Prior and N.T. Slater (1987) *Soc. Neurosci. Abstr.*, 13: 976.

381.14

POTENTIATION BY HALOPERIDOL OF THE ANTAGONISM BY MK-801 OF THE EXCITATORY EFFECT OF DICARBOXYLIC AMINO ACIDS: AN ELECTROPHYSIOLOGICAL STUDY IN THE RAT DORSAL HIPPOCAMPUS. F. P. Monnet*, G. Debonnel and C. de Montigny (SPON: L. Vachon). Institut P. Pinel and McGill University, Montréal, Québec, Canada.

Behavioral and electrophysiological studies have shown that MK-801, an agonist of the PCP_1 receptor, blocks specifically the excitatory and proconvulsive actions of N-methyl-D-aspartate (NMDA).

Male Sprague-Dawley rats were anesthetized with urethane (1.25 g/kg, i.p.). Five-barrelled glass micropipettes were used for extracellular recording and microiontophoresis. The central barrel was used for unitary recording and the side barrels filled with the following solutions: kainate (1 mM in NaCl 400 mM, pH: 8), quisqualate (1.5 mM in 400 mM NaCl, pH: 8), NMDA (50 mM in 400 mM NaCl, pH: 8), and MK-801 (50 mM in 200 mM NaCl, pH: 4).

In both CA_1 and CA_3 regions of the dorsal hippocampus, the suppressant effect of MK-801 on NMDA-induced activation of pyramidal neurons was more than six times greater than its effect on kainate- and quisqualate-induced activations. Haloperidol (2 mg/kg, i.v.) markedly potentiated the suppressant effect of MK-801 on the three excitatory amino acid-induced activations.

These results confirm the selective antagonism of NMDA by MK-801 and suggest an allosteric interaction between the PCP_1 and σ-haloperidol subtypes of receptors.

381.15

STUDIES ON THE EFFECTS OF EXCITATORY AMINO ACID ANTAGONISTS IN THE MEDIAN RAPHE NUCLEUS OF THE RAT. D. Wirtshafter, J. Krebs* & R. Trifunovic*. Dept. Psych., Univ Ill. at Chicago, Chicago, IL 60680.

In previous studies we have shown that injections of a number of excitatory amino acid (EAA) antagonists into the median raphe nucleus (MR), but not adjacent structures, result in marked increases in locomotor activity and food and water intake. In the current experiments, we attempted to clarify the receptor subtypes responsible for these effects.

Locomotor activity was measured after injections of a number of relatively selective competitive NMDA antagonists into the MR, and the threshold doses for the production of hyperactivity were correlated with the affinity of these compounds for the NMDA receptor. The following compounds were examined, in order of behavioral potency: CPP > DL-AP5 = DL-AP7 = Asp-AMP > Glu-AMP > AP6. In contrast AP4, AP8, and L-AP5, which have very low affinity for the NMDA receptor, were without behavioral effect. In contrast, intra-MR injections of the noncompetitive NMDA antagonists PCP and MK-801 were without effect on activity. One explanation of this finding is that NMDA receptors in the MR may not be coupled to PCP receptors. Feeding and drinking in sated rats could also be elicited by injections of the specific NMDA antagonists CPP and AP5, with the former compound being considerably more potent.

Hyperactivity, and feeding, could also be elicited by intra-MR injections of the kainate/quisqualate antagonists pBB-PZDA and GAMS at doses far lower than those expected from their affinity for the NMDA receptor. These results suggest that both NMDA and kainate/quisqualate receptors may play a role in MR function.

381.16

COMPARATIVE ACTIONS OF EXCITATORY AMINO ACIDS ON STRIATAL DOPAMINE AND ENKEPHALIN RELEASE. B.B. Ruzicka*, D.W. Clow* and K. Jhamandas. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, K7L 3N6.

In the present study, the effect of L-glutamate (L-Glu), N-methyl-D-aspartate (NMDA), kainate (KA) and quisqualate (Quis) on the release of endogenous dopamine (DA) and methionine-enkephalin (ME) from slices of the rat caudate-putamen were investigated. L-Glu, NMDA, KA and Quis, in the absence of Mg^{2+}, produced a dose-related, Ca^{2+}-dependent increase in DA release. DA release induced by L-Glu and NMDA was Mg^{2+}-sensitive and antagonized by D-2-amino-7-phosphonoheptanoate (D-APH) (0.5 mM). In contrast to their excitatory actions on DA release, L-Glu, NMDA, KA and Quis, in the absence of Mg^{2+}, had little effect on ME release. L-Glu (10 mM) also failed to influence the 25 mM K^+-evoked release of ME. However, in the absence of Mg^{2+}, the 25 mM K^+-evoked ME release was significantly greater than that observed in the presence of Mg^{2+}. D-APH (0.5 mM) had no effect on the 25 mM K^+-induced ME release in the absence of Mg^{2+}. These results show that activation of excitatory amino acid receptors causes an increase in the release of DA, but no apparent increase in the release of ME.

(Supported by the Medical Research Council of Canada and the Ontario Mental Health Foundation)

381.17

EFFECTS OF QUINOLINIC ACID ON AMINO ACID RELEASE IN THE RAT STRIATUM IN VIVO. B.A. Donzanti and B.K. Yamamoto, Dept. Pharmacol., Northeastern Ohio Univ. Col. of Med., Rootstown, OH 44272

Quinolinic acid (QA) has been hypothetically linked to a variety of neurodegenerative disorders. Removal of the cortico-striatal glutamatergic input prevents QA-induced neurotoxicity. Thus, it is possible that the neurotoxicity occurs via the presynaptic release of other endogenous excitatory amino acids. However, in contrast to kainic acid (KA), in vitro striatal studies have repeatedly failed to substantiate such a mechanism. Using intracranial microdialysis in conjunction with HPLC with electrochemical detection, we have studied the in vivo release of amino acids from the rat striatum. Urethane anesthesized rats were implanted with 3 mm dialysis probes into the medial caudate. A modified Ringer's medium was perfused through the system at a rate of 2.5 ul/min. Twenty min perfusate samples were collected and analyzed for amino acid content by O-pthalaldehyde derivatization. Following a 2 hr baseline stabilization, QA (12 mM) or KA (12 mM) was perfused through the probe for 20 min. QA perfusion increased ASP (252 + 44%) and GLU (226 + 7%) release. In addition, there was also a marked increase in TAU (333 + 62%). Other amino acids did not consistently change. Similarly, but more dramatically, KA induced ASP (421 + 25%), GLU (319 + 54%) and TAU (6714 + 253%) release. In conclusion, these data suggest that QA-induced striatal release of excitatory amino acids (i.e., GLU and ASP) may be responsible for its neurotoxic properties. We are currently repeating these studies in the unanesthesized, behaving rat.

381.18

N-METHYL-D-ASPARTATE RECEPTORS MAY MODULATE DENDRITIC DOPAMINE RELEASE IN RAT SUBSTANTIA NIGRA. G. Bustos, R. Araneda* and J. Abarca*. Lab. of Biochem. Pharmacol., Catholic University of Chile, Santiago, Chile.

A superfusion system was used to study the effects of excitatory amino acids (EAA) on release of [^{3}H]dopamine (DA) previously taken up by rat substantia nigra (SN) slices. The EAA tested (50 to 250 μM), with the exception of quisqualate and kainate, markedly evoked [^{3}H]DA release when Mg^{2+} ions were omitted from the superfusion medium. The EAA receptor agonists exhibited the following relative potency in stimulating [^{3}H]DA release: L-GLU > NMDA = NM(D,L)A > D-GLU >> quisqualate = kainate. Further experiments showed that the NMDA-mediated release of [^{3}H]DA was totally supressed by D-2-amino-5-phosphonovalerate (100-200 μM), or by the omission of Ca^{2+}, or by the addition of TTX (0.1 μM) to the superfusion medium. In addition, strychnine (10 μM) significantly decreased NMDA-evoked as well as glycine-evoked release of [^{3}H]DA from nigral slices. It is suggested that activation of NMDA-subtype receptors in SN may trigger a Ca^{2+}-dependent release of DA from dendrites of nigro-striatal DA-containing neurons. A transynaptic mechanism partially involving glycine-containing interneurons may account for some of the events mediating NMDA-receptor activation and DA release in SN.

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381.19

EVIDENCE AGAINST THE CO-TRANSMITTER ROLE OF GLUTAMATE IN CORTICAL CHOLINERGIC TERMINALS. J.C.Szerb and A.Fine Dept.of Physiol.Biophys. Dalhousie Univ.Halifax, N.S.B3H 4H7,Canada.

Recently Docherty et al.(Nature,330:64,1987) described the isolation of cortical cholinergic and GABA-ergic terminals by means of ChAT or GAD antisera and magnetic microspheres. These terminals contained and released large amounts of glutamate, which suggested that glutamate (GLUT) may have a major role as a co-transmitter in cholinergic and GABA-ergic terminals. To see whether the GLUT content of rat cortex can be influenced by lesions of the cholinergic terminals, as predicted by the co-transmitter role of glutamate, we compared cholinesterase (ChE) activity and GLUT content of the frontal and parietal cortex of the two hemispheres following unilateral injection of ibotenic acid into the n.basalis. While the ChE activity ipsilateral to the lesion was massively depressed 7 days after the lesion, no change in the GLUT content (102% compared to the intact side) was found. Furthermore, a 67% depression by 10 μM oxotremorine of the rate of [^{3}H]ACh release induced by 25 mM K^+ was not accompanied by any change in endogenous GLUT release. Since it is well established that the release of all co-transmitters is regulated by the same autoreceptors (Bartfai et al. Ann.Rev.Pharmacol.28:285, 1988), the lack of co-regulation of the release of ACh and GLUT by muscarinic autoreceptors also suggest that the co-transmitter role of GLUT with ACh must be minor. The occurrence of large amounts of GLUT in isolated cholinergic terminals is likely to be due to contamination with glutamate-ergic synaptosomes. (Supported by the MRC of Canada.)

381.20

MK-801 BEHAVIORAL ACTION AND BLOCKADE OF D_1-DOPAMINE-RECEPTOR PRIMING IN NEONATAL-6-OHDA-LESIONED RATS. **H.E. Criswell, R.A. Mueller and G.R. Breese Univ of North Carolina Sch. of Medicine. Chapel Hill NC 27599**

MK-801 antagonizes NMDA receptor function, enhances locomotor activity in rats, and antagonizes long-term-potentiation. In this work, neonatal- but not adult-6-OHDA lesioned rats were supersensitive to the behavioral activating effects of MK-801. The increased activity induced by MK-801 in neonatal-6-OHDA-lesioned rats was blocked by prior administration of alpha methyltyrosine (40 or 100 mg/kg) indicating that endogenous dopamine is needed for behavioral activation. Even though MK-801 increased activity through a dopaminergic mechanism, unlike other indirect acting agents and a D_1-DA agonist, this drug was unable to prime D_1-DA receptors upon repeated administration. Because the priming of D_1-DA receptors may reflect a long term neural message, the effect of MK-801 on this phenomenon was assessed. The increase in behavioral effects observed in neonatal-6-OHDA-lesioned rats following repeated administration of a D_1-dopamine agonist (priming) was blocked by the concomitant administration of MK-801 at a dose (0.17mg/Kg) which produces behavioral activation by itself or by a higher dose (1 mg/kg) which produced sedation. These results suggest that NMDA receptors influence DA function and can influence priming--a permanent neural message.

(Supported by NS-21345 and HD-23042

382.1

IgG FROM PATIENTS WITH LAMBERT-EATON SYNDROME INHIBITS CALCIUM-ACTIVATED POTASSIUM CURRENTS. Yong I. Kim. Depts. of Neurology and Biomedical Engineering, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

Recent evidence indicates that the antigenic target of Lambert-Eaton syndrome (LES) IgG is a voltage-dependent calcium channel (Kim and Neher, Science 239:405, 1988). The aim of present study is to determine whether LES IgG also modifies Ca^{2+}-dependent potassium channel currents ($I_{K(Ca)}$). Bovine adrenal chromaffin cells (mean C$_m$=8.03 pF) were incubated for 24 hours with IgG (4 mg/ml) from an LES patient with small-cell carcinoma of the lung and healthy control subjects. In mouse passive transfer studies, this patient's IgG was found to block evoked ACh release by 75%. Using patch clamp technique, whole-cell K^+ currents (I_K) were recorded from the cells held at -80 mV, and depolarized to -40 to +120 mV (exposed to 2 mM Ca^{2+} buffer); peak $I_{K(Ca)}$ occurring at +15 to +35 mV was analyzed by the method of Marty and Neher (J. Physiol. 367:117, 1985). In 22 LES IgG-treated cells, $I_{K(Ca)}$ was 178 pA/pF, declining by 51% from the control (361 pA/pF, n=22 cells) and 55% from the normal untreated (398 pA/pF, n=17). By comparison, I_K elicited at +80 mV fell by 20 and 11%, respectively, relative to the control and normal. These results provide evidence for a significant inhibition of Ca^{2+}-activated K^+ channel currents in this disorder. Clinical implication of this finding is yet to be demonstrated (Supported by NIH grant NS18607 and an MDA research grant).

382.2

ELECTROPHYSIOLOGICAL ACTIONS OF THE K^+ CHANNEL ACTIVATORS, BRL 34915 AND PINACIDIL IN GUINEA PIG CARDIAC MUSCLE. J. R. McCullough and M. L. Conder*. Dept. of Pharmacology, Squibb Inst. for Med. Res. Princeton, NJ 08543-4000.

Activation of K^+ channels of vascular smooth cells with resultant hyperpolarization of the resting potential (RP) is thought to underlie the mechanism of action of the novel antihypertensives, BRL 34915 (BRL) and pinacidil (PIN). The actions of BRL and PIN in cardiac cells are not well understood. To this end, we used standard intracellular microelectrode and whole-cell patch-clamping techniques to study the electrophysiological actions of these agents in papillary muscles and isolated ventricular myocytes of the guinea pig. Both BRL (10^{-5}-10^{-4} M) and PIN (10^{-5} - $5x10^{-4}$ M) caused marked shortening of action potential duration (APD) measured at 50 and 90% repolarization; APD was shortened by up to 80% of control with higher concentrations. RP hyperpolarized several mV in the presence of BRL and PIN, with little effects on other parameters. The inhibition of APD by both compounds was completely and rapidly reversed in a dose dependent manner by addition of 10^{-7}-10^{-5} M glyburide, a sulfonylurea known to inhibit ATP-regulated K^+ channels. In isolated myocytes, BRL ($5x10^{-6}$ M) significantly hyperpolarized RP (in 1-10 mM K^+) and increased the slope of the relationship between RP and K^+ from -41 to -55 mV, suggesting an increase in K^+ permeability. Whole cell voltage clamp experiments failed to find an increase in the inwardly rectifying K^+ current responsible for RP with $5x10^{-6}$ M BRL. Higher concentrations of BRL ($5x10^{-5}$-10^{-4} M) increased the outward current positive to -50 mV in some cells, suggesting that this current may be responsible for the APD shortening observed in the multicellular preparations. These results suggest that different mechanisms or channels may underlie the hyperpolarizing and the APD shortening actions of BRL and PIN and that the latter may be mediated through the ATP-regulated K^+ channel.

382.3

STIMULATORY EFFECTS OF 4-AMINOPYRIDINE AND pH ON CALCIUM-ACTIVATED K CHANNELS IN GH_3 CELLS. D.J. Fickbohm* and G.S. Oxford (SPON: A.E. Stuart). The Neurobiology Program, Univ. of North Carolina, Chapel Hill, NC 27599.

4-aminopyridine (4AP) is often used to block voltage-dependent K(V) channels and to distinguish them from Ca^{++}-activated K(Ca) channels which are gnerally insensitive to 4AP. However, it has been reported (Herman & Gorman, J. Gen. Physiol. 78:63, 1981; Rogawski et al., Soc. Neurosci. Abs. 11:790, 1985) that 4AP can increase both macroscopic and single K(Ca) channel currents. The present study confirms and extends these findings in GH_3 cells. Cells were examined using whole cell and single channel patch recording techniques. Macroscopic K(Ca) currents were measured at +60mV following a 1 sec prepulse to 0mV to both promote Ca^{++} entry and inactivate competing K(V) currents. 4AP (2-5mM) increased such currents by 300%, whereas little or no change was observed in the absence of extracellular Ca^{++}. Raising the pH_o to 8.5 elicited a similar, yet smaller, increase in K(Ca) which was dependent on external Ca^{++}. In cell-attached recordings, bath applied 4AP (2-10mM) greatly increased single K(Ca) channel activity under standard conditions, but not in the absence of bath Ca^{++}. Preliminary experiments have revealed that : (1) whole cell (L-type) Ca currents are not enhanced by 4AP, (2) single Ca channel activity in cell-attached patches is not changed by 4AP, and (3) 4AP does not obviously alter the Ca^{++} sensitivity of K(Ca) channels in excised inside-out patches. Supported by NIH NS18788.

382.4

RYANODINE MODIFIES THE ION-SELECTIVE AND ION-TRANSPORT PROPERTIES OF LOCUST MUSCLE POTASSIUM CHANNELS. E. Gorczynska*, P.L. Huddie* and P.N.R. Usherwood*, Department of Zoology, University of Nottingham, Nottingham NG7 2RD.
(SPON. L.D. Partridge).

Giga-ohm seal patch clamping of adult locust muscle revealed 3 types of K^+ channel, which differed with respect to the voltage dependence and kinetics of channel gating and relative open channel conductances (100pS, 20pS, 10pS). A pNa/pK ratio of 0.067 was estimated (using the Goldman, Hodgkin, Katz equation) for the two largest channels. At concentrations of $\geq 10^{-9}$M ryanodine (a plant alkaloid) increased pNa/pK to a value approaching unity. The ion selective and ion transport properties of these potassium channels have been investigated further in the absence or presence of ryanodine using excised inside-out patches of muscle membrane with a variety of cations, viz. Li, Na, K, Rb, Cs. The relative permeabilities of these ions in the presence and absence of ryanodine will be described. The signficance of these data in terms of the molecular properties of the potassium channels and their transport of cations will be discussed.

382.5

PHARMACOLOGICAL CHARACTERIZATION OF SINGLE-CHANNEL K+ CURRENTS IN *DROSOPHILA* MUSCLE. M. Gorczyca, and C.-F. Wu. Dept. of Biology, Univ. of Iowa, Iowa City, Iowa 52242

In *Drosophila* muscle, at least four macroscopic potassium currents can be distinguished by differences in their kinetic and pharmacological properties and by their sensitivity to a number of mutations. Studies of single channel events underlying these currents have only recently been initiated in muscle membrane vesicles and cultured myotubes. We now report single channel currents observed in body wall muscles *in situ* in wild-type larvae.

Using the inside-out patch clamp configuration, we have observed several distinct potassium channel types. The most prominent one was calcium-dependent, becoming activated in the concentration range of 10^{-7} to 10^{-6} M. The channel displayed a very high open probability at 10^{-7} M and greater, had a conductance of 70-80pS (in symmetrical 130K+), and was not sensitive to 50mM TEA, 1mM 4A-P, and 0.1mM quinidine. It was blocked by barium at concentrations greater than 0.1mM. A second channel type with a 12pS (130K+ pipette/2K+ bath) conductance, was partially blocked by 10mM TEA, 0.1mM quinidine, and 0.1mM barium but not by 1mM 4A-P. A third channel type shared the pharmacological profile of the 12pS channel but its conductance was twice as large. Barium caused a slow block in these three channel types whereas TEA and quinidine led to a fast block. A fourth channel type was highly sensitive to TEA, being almost completely blocked at 1mM. We are currently attempting to correlate these single channel conductances to the previously described macroscopic currents.

382.6

OPEN CHANNEL NOISE IN CALCIUM-ACTIVATED POTASSIUM CHANNELS. M.I. Glavinović, Dept. Anaesthesia Research, Physiology and Biomedical Engineering Unit, McGill University, Montréal, Québec, Canada.

The current through an open ion channel is carried by discrete ions and is thus expected to show fluctuations due to a 'shot noise' (Schottky, Ann. Phys. 57, 541-567, 1918), and as recent studies in ACh receptor channels (Sigworth, Biophys. J. 47, 709-720, 1985) have shown due to fluctuations in the conformation of channel protein also.

In this study the single channel currents were recorded from a calcium activated potassium 'maxi' channels in excised inside-out patches of mouse spinal cord neurons and bovine chromaffin cells at room temperature (20-22° C). The variance of the open-channel current exceeds that of the background; shot noise cannot account for the difference. The low-frequency component of the power spectrum (corrected for the background noise and transfer function of recording system) of the open channel noise could be well fitted with a single Lorentzian. It appears to arise from fluctuations in channel conductance of $\sim$ 3% on a time scale of < 0.2 msec arising presumably from conformational fluctuations in the channel protein. The characteristic relaxation time of the low frequency component showed only a marginal voltage dependence, but its variance was altered when current amplitudes changed with changing membrane potential. Fluctuations in the conformation of channel protein appear to be characteristic of each type of ion channel. Supported by MRC (Canada).

382.7

J-CURRENT: A RAPIDLY RECTIFYING CALCIUM-DEPENDENT OUTWARD CURRENT MECHANISM Daniel K. Hartline, Donald V. Gassie*, Beverly A. Tomiyasu*, and Bradley R. Jones. Békésy Lab, Univ. of Hawaii, Honolulu, HI 96822

A transient, calcium-dependent outward current (I_J) has been described in somata of neurons of the stomatogastric ganglion in crustaceans (Graubard and Hartline Soc. Neurosci. Abstr. 10:1073; Hartline et al. ibid 11:1023). Study of tail currents shows that:

1. I_J activates between -25 and 0 mV.
2. I_J tails reverse near -60 mV in normal saline (13 mM K^+).
3. Tail reversal is dependent on $[K^+]_o$ but less so (30 mV/decade) than would be expected of a simple K^+ mechanism.
4. Plots of $\exp(E_J F/RT)$ vs $[K^+]_o$ are linear, but do not go through the origin.
5. I_J tail reversal level is not obviously affected by changes in external Na^+, Cl^-, or pH.
6. Current jumps during step-back protocols are consistent with an instantaneously or rapidly developing rectification. Conductance near 0 can be 10-fold greater than near -50 mV.

Supported by NIH grant NS15314 (DKH) and a grant from the Ida Russell Cades fund (BRJ).

382.8

A SLOW, AMINOPYRIDINE-SENSITIVE, TRANSIENT K^+ CURRENT IN FROG AUTONOMIC GANGLION NEURONES. J.A. Zidichouski*, P.A. Smith & A.A. Selyanko*, SPON: R.J. Walker, Dept. Pharmacol., Univ. Alberta, Edmonton, Canada & Bogomoletz Inst. Physiol., Kiev, U.S.S.R.

The whole-cell patch clamp technique was used to study K^+ currents in neurones dissociated from Rana pipiens autonomic ganglia. Membrane repolarization to -60 to -30mV from hyperpolarizing voltage commands to -60 to -140mV revealed classical, fast, transient, outward tail currents (I_A, A-currents) in paravertebral sympathetic ganglion B-cells but not in cardiac parasympathetic or sympathetic ganglion C-cells. In all three neurone types however, our methodology revealed another, hitherto undescribed, transient, Ca^{2+} independent K^+ current (I_{SA}) which exhibited a similar voltage dependence to I_A yet activated and inactivated with a 100-fold slower time course. I_{SA} slowly developed during the course of whole cell patch clamp experiments and was antagonized by 2mM 4-AP but not by Ba^{2+} (2-4mM), TEA (3mM) or d-tubocurarine (70μM). Muscarine (10μM) rapidly and reversibly induced I_{SA} in both B- and C- sympathetic ganglion cells but inhibited it in parasympathetic cells. Control of I_{SA} by neurotransmitters may therefore be important in the modulation of neuronal repetitive discharge characteristics.

Supported by MRC and AHFMR.

382.9

INWARD RECTIFICATION IN MAMMALIAN CNS AXONS. D.L. Eng,* T.R. Gordon, J.D. Kocsis, and S.G. Waxman. Neuroscience Research Lab, VA Medical Center, West Haven, CT, 06516.

Inward rectification has been studied in a variety of neuronal systems. Rat optic nerves were studied in a modified sucrose gap using whole nerve current clamp of the axonal membrane. Hyperpolarizing constant current pulses led to a delayed depolarizing sag corresponding to decreased membrane resistance. This inward rectification was present in TTX, 4-AP, and TEA, indicating that it is not dependent on ion channels sensitive to these agents. CsCl, known to block Na^+ and K^+-dependent inward rectifiers in other systems, blocked the inward rectifying conductance in rat optic nerve. $BaCl_2$, a blocker of K^+-dependent inward rectifiers, reduced the inward rectification in rat optic nerve. The inward rectification was absent in Krebs' solution containing choline substituted for K^+ and Na^+. The reintroduction of Na^+ or K^+ led to partial restoration of the inward rectification, with Na^+ being more potent than K^+. The results indicate that inward inward rectification is present on a CNS axon system, the rat optic nerve, and is due to a mixed Na^+/K^+ conductance.

382.10

PATCH-CLAMP RECORDED CHANNELS IN PLASMA CELLS ISOLATED FROM AN AVIAN LACRIMAL GLAND. B. Walcott, S.F. Fan* E. Roemer*and P. Brink. Department of Anatomical Sciences, State Univ. of New York, Stony Brook, N.Y. 11794.

Avian lacrimal glands contain large populations of plasma cells that secrete the immunoglobulins found in high concentrations in the tear fluid. Those regions of the glands particularly rich in lymphocytes are densely innervated by cholinergic and noradrenergic fibers of the autonomic nervous system and there is extensive substance P-like and vasoactive intestinal polypeptide-like immunoreactivity as well. Physiological experiments have shown that carbachol increases the release of total protein and immunoglobulins from isolated gland fragments. Thus there is the possibility of neural modulation of the secretory immune response. To examine this directly, we have begun to characterize the channels found in isolated plasma cells with the intention of determining the effects of neurotransmitters on them. Glands dissected from chickens were carefully cleaned of adhering fat and then were teased apart in RPMI media. The isolated cells were centrifuged once in RPMI, replated and treated with 0.1 mg/ml collagenase for 20 minutes. Morphologically identified plasma cells were patch-clamped and showed large numbers of voltage dependent K^+ channels that were also Ca^{++} dependent.

382.11

PATCH-CLAMP RECORDINGS FROM DISPERSED TURTLE RPE CELLS, by J. A. Fox and R. H. Steinberg, Departments of Ophthalmology and Physiology, U.C. San Francisco, San Francisco CA 94143.

The electrophysiological properties of isolated turtle (*Pseudemys scripta elegans*) retinal pigment epithelial cells (RPE cells) were investigated using the patch-clamp technique. Cells were dispersed with papain and plated onto glass cover-slips for patch recording with fire-polished pipets (Corning 8161 glass, 2-5 MegOhms) under constant flow of solution bubbled with 95 % O2 - 5 % CO2. The bath contained (mM): 115 NaCl, 25 Na HCO_3, 10 dextrose, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, pH 7.3. Pipet solutions containing 135 mM KF or KCl, 15 $KHCO_3$, 0.5 mM $CaCl_2$, 5 mM EGTA, and 5 mM $MgCl_2$ were bubbled and then filtered before use.

The average resting potential of 67 cells was -36 ± 20 mV, with average whole-cell capacitance of 85 ± 24 pF. Virtually all cells had outwardly rectifying I-V relations. Most cells exhibited a voltage dependent outward current activated by depolarization beyond about -30 mV, which inactivated during a 500 ms voltage step. This current exhibited steady-state inactivation, being much reduced or absent when cells were held at -30 mV. It was abolished by 500 μM quinidine and was reduced by 100 μM quinidine, 2 mM Ba, 2 mM TEA and 2 mM 4AP. Reversal potentials for this current, as measured by tail currents in 10 cells, were -70 ± 9 mV in normal (3 mM KCl) ringer's and -36 ± 5 mV in 30 mM KCl ringer's, indicating potassium selectivity. Many cells had slowly activating outward currents upon depolarization beyond about + 40 mV which appeared to reverse near 0 mV in both 3 mM KCl and 30 mM KCl solutions. This current was not greatly affected by the blockers which affected the K+ current. Less frequently we observed activating or inactivating (during a 500 ms voltage step) inward currents at potentials more negative than -70.

382.12

CALCIUM-ACTIVATED POTASSIUM AND CHLORIDE CURRENTS IN RABBIT PARASYMPATHETIC NEURONS. T. Tokimasa, T. Nishimura*, M. Tsurusaki* and T. Akasu*. Dept. of Physiology, Kurume Univ. Sch. of Med., Kurume 830, Japan.

Vesical pelvic ganglion cells of the rabbit urinary bladder were voltage clamped using a single microelectrode in a Krebs solution containing TTX (1 μM). Inward rectification at potentials negative to -80 mV was blocked by 2 mM cesium ions. Calcium-activated potassium current which underlies the spontaneous hyperpolarizing oscillations of the resting membrane and the action potential after-hyperpolarization was selectively blocked by apamin (1-10 nM) and curare (30-300 μM). Superfusing the preparation with a nominally calcium-free solution eliminated not only these calcium-activated hyperpolarizations but also the persistent potassium current which was already activated at the resting potential of about -60 mV. The persistent potassium current was insensitive to apamin and curare but was non-selectively blocked by 20 mM TEA and intracellular injection of Cs ions or EGTA. Under the complete suppression of the calcium-activated potassium current, the inward calcium current evoked by commanding the cells from -60 mV to -10 mV for 50-100 ms was followed by an inward tail current. The inward tail was due to an efflux of chloride ions. Both calcium and chloride currents were blocked in a calcium-free solution.

382.13

WHOLE-CELL VOLTAGE-CLAMP STUDY OF TWO DISTINCT COMPONENTS OF "A CURRENT" IN HIPPOCAMPAL NEURONS. B.E. Alger and D. Doerner, Dept. Physiol, Univ. MD. Sch. Med., Baltimore, MD 21201.

Using whole-cell voltage-clamp techniques we identified two distinct transient outward currents in hippocampal neurons acutely dissociated from adult guinea pig or tissue cultured from fetal rat. In control saline with 10-20 mM TEA, depolarizing steps from relatively hyperpolarized holding potentials elicit a rapidly-activating K current which inactivates fully with a time constant of 60-100 ms. 4-AP (1-10 mM) reduces total transient current, unmasking a component which inactivates much more rapidly, with a time constant of 10-20 ms. The 4-AP-insensitive transient current is completely blocked by 100-200 μM Cd or by low Ca saline. Conversely, addition of Cd to the control saline reduces total transient current and slows the overall time constant of inactivation. In Cd, the residual transient current, corresponding to the A current (I_A), was blocked by bath application of 4-AP. Both transient current components showed similar voltage-dependence.

The contribution of I_A and the Cd-sensitive current to total transient current was variable. I_A was ubiquitous and invariably larger than the Cd-sensitive transient in acutely isolated adult neurons. The faster-inactivating, Ca-dependent transient was larger and more prevalent in cultured neurons from fetal rat hippocampus.

382.14

SINGLE ELECTRODE WHOLE CELL VOLTAGE AND CURRENT CLAMP ANALYSIS OF POTASSIUM CHANNELS IN HAIR CELLS ISOLATED FROM THE CRISTA AMPULLARIS OF THE FROG. G.D. Housley[1], C.H. Norris[1,2] and P.S. Guth[1]. Depts. [1]Pharmacology & [2]Otolaryngology, Tulane University, New Orleans, LA 70112.

Hair cells from the crista ampullaris of the frog (*Rana pipiens*) were isolated enzymatically (0.17 mg/ml papain in low Ca^{++} artificial perilymph) and placed in short-term culture (Leibovitz L-15 medium; Sigma). The resting membrane potentials (V_z) were -44.9 ± 1.5 mV (x ± SEM; n=51). Two types of outward current were identified corresponding to the fast transient K^+ current (K_A) and the slower Ca^{++} dependent K^+ current (K_{Ca}) reported by Lewis and Hudspeth (Nature 304:538-541, 1983). Using a conditioning pulse protocol; most inactivation of the K_A current occurred between -60 mV and -10 mV, with 50% inactivation occurring with conditioning pulses close to V_z. The K_A channels inactivate exponentially with a time constant of about 16 ms. Tetraethylammonium (TEA; 10 mM) blocked only the K_{Ca} current. Replacing the K^+ (103 mM) with equimolar Cs^+ in the internal solution abolished the outward current and left only a small inward current. The mean K_A and K_{Ca} conductances (from -20 mV to +20 mV) were (x ± SEM) 15.8 ± 3.96 nS and 21.3 ± 1.60 nS respectively (n=7). Injecting 1 nA of current produced damped oscillations in cell membrane potentials. This resonance, only previously reported in hair cells of putative auditory function (Crawford and Fettiplace, J. Physiol. 312:377-412, 1981; Lewis and Hudspeth, Nature 304:538-541, 1983; Pitchford and Ashmore, Hear. Res. 27:75-83, 1987), is thought to be the result of an interplay between an inward Ca^{++} current and the outward K_{Ca} current. TEA blocked this resonance. Support: N.I.H.-NS 22051; Southern Hear. & Speech Fndn.; N.Z.DRF; MRC(N.Z.).

382.15

COMPARISON OF 4-AMINOPYRIDINE (4-AP) AND TETRAHYDROAMINO-ACRIDINE (THA) ON MEMBRANE PROPERTIES AND IONIC CURRENTS IN GUINEA PIG BASAL FOREBRAIN. J.A. Sim* and W.H. Griffith (Spon: L.L. Keeley). Dept. of Medical Pharmacology and Toxicology, College of Medicine, Texas A & M University, College Station, TX 77843.

The actions of 4-AP and THA were studied in basal forebrain (BF) neurons in vitro using intracellular recording and single electrode voltage-clamp (SEVC) techniques. Both compounds decreased several presumed potassium (K^+) conductances, but over different concentration ranges. In all cell types, 4-AP (100-300 μM) hyperpolarized the membrane potential, increased membrane conductance, prolonged action potential duration and increased spontaneous transmitter release. Opposite membrane effects were elicited in all cell types by THA (100-300 μM). A transient outward current (A-current) was described in all three cell types. Both 4-AP (100 μM) and THA (300 μM) reduced the A-current by approximately 50% with no change in voltage dependence. Blockade was concentration dependent and readily reversible with 4-AP. THA, but not 4-AP, had an additional effect to block time-dependent inward rectification in F-AHP cells. Despite structural similarities between the two compounds, our results demonstrate numerous differences between 4-AP and THA when compared in BF neurons. Supported by NIH Grant NS22456 (WHG) and the U.K. Medical Research Council (JAS).

MUSCLE: FUNCTION AND BIOCHEMISTRY

383.1

THE INFLUENCE OF INTERMITTENT ELECTRICAL STIMULATION ON THE MECHANICAL PROPERTIES OF A PREDOMINANTLY FAST MUSCLE IN ADULT AND SENESCENT FISCHER-344 RATS. T.J. Walters*, H.L. Sweeney and R.P. Farrar(SPON: P. MacRea). Dept. of Kinesiology, Institute for Neurolgoical Sciences Research. Univ. of Texas, Austin, TX 78712.

The influence of elevated neural activity on the mechanical properties of the predominantly fast flexor digitorum longus (FDL) was investigated in adult (7-8 mo.) and aged (27-28 mo.) Fischer-344 rats using intermittent electrical stimulation. The FDL was stimulated via an electrode cuff around the tibial nerve at a frequency of 10 Hz for 8 hr/day for periods ranging from 0-90 days (0, 20, 35, 60, and 90 days). Both age groups displayed an increase in the twitch tension/tetanic tension (P_t/P_o) following 20 days of stimulation, with no significant increase at subsequent time points. The increase in P_t/P_o was a result of both an increase in P_t and a decrease in P_o. There was no significant differences between the two ages at any of the corresponding time points. A significant increase in the time to peak twitch tension occurred by 20 days in aged rats (14.03 msec vs. 17.60 msec) and by 30 days in adult rats (15.34 msec vs. 17.64 msec). No subsequent significant increase in TPT occurred after these points. There was no significant difference between any of the corresponding time points between the two ages. The maximal velocity of unloaded (V_{max}) was determined using the slack test. Although an increase in V_{max} occurred at 90 days in both adult rats (248 mm/sec vs. 211 mm/sec) and aged rats (232 mm/sec vs. 169 mm/sec), it was significant only in the aged rats. The fatigue resistance of the FDL was determined using the Burke fatigue test. A progressive significant increase in fatigue resistance occurred through 30 days of stimulation in both ages, at which point a plateau was reached. These data demonstrate that the mechanical properties of the predominantly fast FDL remain remarkably plastic in response to an extreme elevation of neural activity through very old age in Fischer-344 rats.

383.2

THE INFLUENCE OF INTERMITTENT ELECTRICAL STIMULATION ON THE BIOCHEMICAL PROPERTIES OF A PREDOMINANTLY FAST MUSCLE IN ADULT AND SENESCENT FISCHER-344 RATS. R.P. Farrar, T.J. Walters, and H.L. Sweeney Dept. of Kinesiology, Institute for Neurolgoical Sciences Research. Univ. of Texas, Austin, TX 78712.

The plasticity of muscle has been inferred to decline during senescence. Previously we (Cartee and Farrar, 1987) demonstrated that the gastrocnemius of young and old rats, given the same aerobic stimulus imposed by running for an hour per day, at the same absolute workload responded with the same absolute increase in aerobic capacity. The purpose of this study was to determine whether an imposed neuronal stimulus, to a muscle with predominantly fast motor units would induce similar changes both in aerobic capacity and fiber type in young adult and senescent rats. We imposed an altered stimulus to the plantaris muscles in adult (7-8 mo.) and aged (27-28 mo.) Fischer 344 by stimulating the tibial nerve at a frequency of 10 Hz for 8 hr/day for periods ranging from 0-90 days (0, 20, 35, 60, and 90 days). The mass of the plantaris muscle in both adult and aged rats decreased 20-25% following 20 days of stimulation and did not change significantly after that. The aerobic capacity, as measured by citrate synthase, of the plantaris muscle increased 3-fold by day 20 of stimulation and dropped down to approximately a 2-fold increase by day 35 of stimulation and remained there throughout the remaining periods of stimulation. There was not a shift in fiber type, as determined by gel electrophoresis on native myofibrillar proteins, until 60 days of stimulation. The shift in fiber type occurred in the fast population with an increase in IIa fibers and concomitant decrease in IIb fibers of approximately 20%. The time course of change in either aerobic capacity or fiber type did not vary between adult and aged rats. These data demonstrate that the plantaris muscle, which has predominantly fast motor units, maintains the same degree of plasticity in the senescent rat as that of young adult rat, when a neuronal stimulus, similar to that of a slow muscle, is imposed for up to 90 days.

383.3

EFFECTS OF CYCLICAL PASSIVE STRETCH IN MAINTAINING CAT SOLEUS MECHANICAL PROPERTIES. R.R. Roy, D.J. Pierotti*, K.M. Baldwin* and V.R. Edgerton. Brain Research Institute and Kinesiology Dept., UCLA, LA, CA 90024 and Physiology Dept., UC Irvine, Irvine, CA 92717.

Four adult female cats had the lumbar region of their spinal cord functionally isolated (SI) by transecting the cord at T12-T13 and at L7-S1 and cutting all dorsal roots bilaterally between these two cord segments. The cats were maintained in excellent health for 6 months. One limb in each cat was exercised passively through a range of motion mimicking a step cycle for 30 min/day, 5 days/week. During the week prior to physiological testing, two 24-hour EMG recording sessions were used to verify that the muscles in the lower limb were virtually silent and recordings from a force transducer on the soleus tendon showed that up to 1.0 kg of force was generated during the passive exercise. In 3 of 4 cats, the soleus muscles in both limbs of the SI cats were significantly smaller (wet weights), had faster twitch contraction times and maximum rates of shortening (Vmax), produced less tetanic tension (Po) and were somewhat less fatigue resistant than the soleus from control cats. When comparing the two limbs within an SI cat, the soleus in the passively exercised limb was 10-30% larger, had a Po that was 28-42% higher and, although more variable, a 20-60% lower Vmax than the soleus in the unexercised limb. All other parameters were similar in the two legs. Adaptations in the myosin ATPase activities and isozyme patterns were generally consistent with the changes in Vmax. These data indicate that short periods of cyclical passive stretching that results in a significant amount of force at the muscle tendon may have a beneficial effect in maintaining the mass and the mechanical properties of electrically silent mammalian muscles. Supported by NIH Grant NS16333.

383.4

METABOLIC AND CONTRACTILE PROTEIN EXPRESSION IN SINGLE MUSCLE FIBERS OF THE SNAKE. P. M. Nemeth, H. L. Sweeney, & R. S. Wilkinson. Depts. of Neurology and Cell Biology, Washington Univ. Sch. of Med., St. Louis, MO 63110, and Dept. of Kinesiology, Univ. of Texas, Austin TX 78712.

Single fibers from the thin transversus abdominis muscle of the garter snake were identified by physiological criteria as faster twitch (F), slower twitch (S) or tonic (T) (Wilkinson and Lichtman, J. Neurosci., 1985). Fibers were subjected to either (1) microchemical analyses for glycolytic (lactate dehydrogenase), oxidative (malate dehydrogenase, fumerase, B-hydroxyacyl CoA dehydrogenase) and high energy phosphate (adenylokinase, AK) enzymes or (2) denaturing SDS discontinuous PAGE for myosin heavy chain composition.

F fibers had high glycolytic and AK activities, and contained one myosin heavy chain isoform similar to rat fast (IIB) myosin. S fibers had high glycolytic and oxidative activities, intermediate AK activity, and expressed two myosin isoforms, one similar to rat slow (I) and the other to rat fast. T fibers had high oxidative activity with low levels of AK; they expressed a single myosin isoform similar to rat slow. The three fiber groups distinguished by metabolic criteria match those of the rat hindlimb; the same groups are distinguished by myosin isoform composition. Thus energy generating enzymes and myosin isoforms strictly correspond in this reptilian muscle. Supported by NIH grants DK38375, AR35661, NS24752 and the MDA.

383.5

RECOVERY OF FORCE PRODUCTION AND FORCE SENSATION FROM FATIGUE. E. Cafarelli and J. Layton-Wood*. Dept. of Phys. Educ., Fac. of Sci., York Univ., Toronto, Ont., M3J 1P3.

Our purpose was to compare the recovery time course of force producing capacity with the recovery time course of force sensation following fatigue. Ten subjects participated after giving their informed consent. During each experimental session fatigue was induced in the right anterior thigh muscles by holding 50% of maximal voluntary contraction force (MVC). This contraction ended when force fell to 20% MVC. In half the experiments a pressure cuff was inflated around the fatigued leg during recovery. The recovery of force production was assessed with: 1) periodic MVC's and 2) the EMG-force ratio during submaximal contractions. Force sensation measurements at 20-50% MVC were obtained using the matching technique (Cannon, R. and E. Cafarelli, J. Appl. Physiol. 63:2396, 1987). In the non occluded experiments, MVC recovered to 80% of control after 3 min but no further. In comparison, force sensation recovered to control after 1 min. Occlusion caused a 10-fold increase in force sensation after 1 min of recovery and a continuous decrease in MVC and EMG in the fatigued leg. When occlusion was removed there was a gradual increase in force producing capacity and a more rapid decrease in force sensation. These results indicate that force sensation recovers somewhat independently of force production and responds to central feed-forward information during recovery from fatigue.
Supported by NSERC A6633

383.6

RECURRENT INHIBITION DURING SUSTAINED SUBMAXIMAL CONTRAC_ TION IN HUMANS. *N. McNabb*, J.S. Frank and H.J. Green*. Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.*

This study examined whether increasing recurrent inhibition to active motoneurons could contribute to the reduction of motor drive during sustained submaximal contraction. Subjects (N=6) performed two different fatigue conditions involving sustained isometric contraction of the plantar flexors: constant torque and constant soleus emg (linear envelop) maintained at 60% MVC. Recurrent inhibition was examined at 3-second intervals throughout the sustained contraction by a H-reflex method developed by Hultborn and Pierrot-Deseilligny (J. Physiol., 297:229, 1979). The method involves comparing the amplitude of a reference H-reflex (Href) with that of a H-reflex preconditioned by recurrent inhibition (H'). H-reflexes were elicited in the soleus muscle by stimulation of the tibial nerve. Fatigue was confirmed by a 20% drop in MVCs performed periodically during the contraction. The test of recurrent inhibition revealed relatively greater reductions in H' than Href for 5 subjects in the constant torque condition and 3 subjects in the constant emg condition. For these subjects, H'-Href values dropped by 10% Mmax within 10s and 20-30% Mmax within 20-50s of voluntary contraction. The greater drop of H' versus Href when voluntary emg is constant or declining indicates increasing recurrent inhibition to the soleus motoneurons during sustained contraction. (Supported by NSERC.)

383.7

STERNOCLEIDOMASTOID MUSCLE INHIBITION INDUCED BY TRIGEMINAL REGION STIMULATION. P.A. Browne*, G.T. Clark and M. Nakano*. UCLA Dental Research Institute, Los Angeles, CA 90024 & Children's Hospital and Chapman College, School of Physical Therapy.

Research suggests a high degree of coupling between trigeminally and cervically innervated muscles. This study documented the presence of co-inhibition in the sternocleidomastoid (SCM) and masseter muscle when using mechanical and painful electrical stimuli applied to the oral and perioral region. Electromyographic activity of the masseter and SCM muscles was recorded bilaterally with surface electrodes during the following conditions: maximal clenching and maximal neck flexion or rotation. The inhibitory stimuli involved menton taps, electrical stimulation intraorally to the anterior maxilla gingiva or extraorally to the skin over the mental nerve. The subjects all demonstrated clear masseter and SCM inhibition of similar timing with menton tap. It is very likely that this inhibition represents a response of the SCM to stretch caused by the direct mechanical perturbation from the tap. In contrast, SCM inhibition with electrical stimulation though often elicited, was not a consistent response. It was not elicited in all subjects nor in every trial of subjects in whom it was elicited. The SCM inhibition was similar in timing and character to the masseter inhibition (e.g., it was frequently a double silent period).

383.8

THE FORCE-VELOCITY AND FORCE-LENGTH BEHAVIOR OF SINGLE MOTOR UNITS OF THE MEDIAL GASTROCNEMIUS MUSCLE OF THE CAT. C.J. Heckman, J.L.F. Weytjens and G.E. Loeb. Lab. of Neural Control, NIH, NINCDS, Bethesda, MD 20892.

Forces developed by single motor units have been studied in reduced preparations (by stimulation of either motoneuron cell bodies or axons), and in humans (by spike-triggered averaging techniques). Detailed information on the mechanical behavior of motor units is available for isometric conditions only. We set out to study the dependence of motor unit twitch and tetanic forces on both muscle length and rate of change of muscle length.

The medial gastrocnemius muscles of 15 cats anesthetized with pentobarbital were separated from the surrounding muscles and their tendons attached to a servo-controlled muscle stretcher through a custom-built force transducer. Precautions were taken to prevent the tendons from drying out. All other hindlimb muscles were denervated. Laminectomies were performed and single motor units were functionally isolated by splitting either the L7 or S1 ventral roots. With this set-up, motor unit twitch forces and tetani at various frequencies were studied at different muscle lengths and during movements at velocities up to half a muscle length per second in both the lengthening and shortening directions. Stimuli were not applied to the units until the velocity of movement was constant.

Analysis of 16 units (5 type S, 4 type FR and 7 type FF) so far showed that 1) with increasing frequency of stimulation the maximum of the force-length curve shifted towards shorter muscle lengths, 2) the force-velocity curves for both single twitches and tetani were "classically" shaped, i.e., forces decreased with shortening and increased with lengthening, 3) the amplitudes of the isometric twitches (zero velocity), however, were heavily dependent on mechanical history: immediately following (100 ms) 2 mm stretches, they were up to 4 times larger than after 10 s or more, 4) the increase in force with lengthening tended to be greater for type S units than for type FR and type FF units.

For further analysis we are presently investigating analytical techniques for separating the velocity effect from the length effect.

383.9

MODULATORY EFFECT OF ZERO SODIUM MEDIUM ON CONTRACTION OF BUCCAL MUSCLE OF APLYSIA. J.L. Ram, G.N. Noirot*, M.A. Anderson*, M.J. Cichowlas*, S. Patel*, & M. Harris*. Dept. of Physiology, Wayne State Univ., Detroit, MI 48201.

Acetyl choline (ACh) depolarizes and causes contractions of Aplysia buccal muscles. Expts. with zero sodium (ONa, tris-substituted) and zero calcium media showed that ACh depolarization was both sodium- and calcium-dependent. ONa hyperpolarized muscles 2-6 mV and reduced ACh depolarzations to 20%-50% of control. ACh depolarization recovered within seven min in normal medium (ASW).

ACh elicited contractions were reduced in ONa. Upon return to ASW, ACh contractions partially recovered within 2 min and were subsequently potentiated. Maximal potentiation after 1, 2, & 10 min in ONa occurred within 4 min of returning to ASW and were 130 ± 10% (n=17), 140 ± 10% (n=19), & 600 ± 130% of control, respectively (all $p<.05$). Time constants for recovery were about 10 min.

ONa (10 min) increased Ca-45 influx from 350 ± 70 (n=7) to 1250 ± 200 (n=7) cpm/mg protein ($p<.05$,t test).

ONa may cause Ca influx via Na-Ca exchange. Additional Ca in sarcoplasm or sarcoplasmic reticulum could enhance responses to Ca entering in response to ACh. Saponin-skinned Aplysia muscle fibers contracted in response to Ca. Raising the "background" level of Ca in perfusion medium increased the contractile response to Ca pulses, thereby modeling this proposed mechanism of modulating contraction. (supported by MDA and NIH grant RR-08167).

383.10

THE EFFECT OF EXTRACELLULAR MATRIX COMPONENTS ON THE SHAPE AND PROLIFERATION OF SKELETAL MUSCLE MYOBLASTS. H.R. Stephens*, C. Lebrecque*, S. Woerly and J.P. Tremblay. Lab. Neurobiol.,Hop. Enfant-Jésus, Univ. Laval, Québec, Canada, G1J 1Z4.

The importance of the extracellular matrix in muscle cell growth and differentiation is well established. In the present 'in vitro' study, we investigated the effect of various extracellular components incorporated with a collagen-based substratum on 1)the cell shape and 2)the growth of the G-8 mouse myoblast line. The six substrata tested included films of dried type I collagen (Vitrogen 100) in 1)non-fibrillar or reconstituted fibrillar form either 2)alone or in combination with 3)hyaluronic acid (5 % wt.) and fibronectin (1% wt.), with 4)chondroitin-6-sulfate (10% wt.) as well as 5)gelatin (1%) and 6)non-coated polystyrene flasks. In addition, the myoblasts were suspended in three-dimensional collagen gels. The morphology of the cells was examined by phase and scanning microscopy; cell kinetics were measured by incorporation of bromodeoxyuridine and cell numbers were recorded at 1, 4, 8 and 14 days post-plating. The proliferation rate of the myoblasts on the fibrillar collagen films was markedly reduced in comparison with non-fibrillar films or gelatin-coated or plastic flasks. In general, the myoblasts were homogeneously distributed and well spread on the non-fibrillar films. In contrast, on the reconstituted collagen lattices they exhibited a wide range of shapes according to whether the cells were embedded within the fibrils (extensive branching cellular processes) or strode the matrix (network of bipolar, spindle-shaped myoblasts aligned end-to-end). There were also differences in the branching and spacing pattern of the myoblasts depending on the presence or absence of the glycosoaminoglycans within the collagen-based extracellular matrix. These results are consistent with reports demonstrating that the spatial configuration, as well as the chemical nature of the extracellular matrix, set the course of the cell towards differentiation rather than cell proliferation.

383.11

INTERSPECIES COMPARISON OF CARDIAC MUSCLE GANGLIOSIDES. K.C. Leskawa* and C.S. Short* (SPON: L.A. Carr). Dept. Anat. Sci. & Neurobiol., University of Louisville, Louisville, KY 40292.

Ganglioside content of hearts from a number of different species was examined, since studies of glycolipids in non-neuronal, electrically excitable tissues are rare. Atria and surrounding adipose tissue were removed and extracted gangliosides were fractionated into mono-,di-, and oligosialosyl forms on DEAE-Sephadex.

Brains of lower vertebrates contain a higher content of complex, oligosialosyl gangliosides; in contrast such phylogenetic patterns were not apparent for cardiac gangliosides. The highest content of di- and oligosialosyl gangliosides was found in the hearts of sheep, horse and pig; human values were slightly lower. Complex gangliosides were much lower in eel, tuna and turtle hearts and lowest in rodent hearts (mouse, guinea pig, hamster and rat).

While calf heart contained numerous complex structures, adult bovine hearts contained only GM3 (99.3% of total). The simplification of cardiac gangliosides with aging is not a generalization, since young and mature rabbit hearts displayed only slight pattern changes although total ganglioside content was reduced.

The remarkable species differences demonstrated by cardiac gangliosides suggests a role in heart function and/or development. (Amer. Heart Assoc., Kentucky Affil.)

383.12

MEASUREMENT AND FOCUSSING OF MAGNETIC STIMULI APPLIED TO NEURONAL STRUCTURES R.G. Bickford, P. Fortescue, A. Rivero* and D. Brittain* Lab of Neurophysiology, University of California at San Diego, 92037

The high frequency magnetic pulses (100 microsecond duration) employed in magnetic stimulation of the nervous system (nerves, spinal roots and cerebral cortex) are of sufficient frequency to give rise to a "skin effect" when applied to a metal surface. We have employed the induced field on the inside of a copper cone to compress and concentrate the magnetic field produced by the coil of a magnetic stimulator (e.g. Cadwell). This allows the production of a highly concentrated field at several centimeters from the coil producing the focussing of the field at its exit.

Tests were carried out using a 1 cm current loop connected to a peak reading voltmeter calibrated in kilogauss (designed by Dr. Fortescue). In one such test the field at 5 cm from the coil read 0.4 Kgauss at 5 cm (at apex). When a 16 gauge copper cone (with a diagonal saw cut to prevent current flow) was put in place centering on the magnet coil, the reading was increased to 1.4 Kgauss indicating a tenfold concentration. The clinical features of the focussed field was investigated in the isolated frog muscle and by application to a subject's cutaneous nerves (ulnar, median and digital). EMGs resulting from the experiments will be shown.

383.13

FLUCTUATING THRESHOLDS IN HUMAN MOTOR AXONS

J. Daube. Division of Clinical Neurophysiology, Mayo Clinic, Rochester, MN 55905

Percutaneous, submaximal, electrical stimulation of mixed nerves in 30 healthy human subjects resulted in random fluctuation of the amplitude of the compound muscle action potential (CMAP). These fluctuations varied with skin temperature and sympathetic activation. The distributions of the CMAP amplitudes were approximately Poisson near CMAP threshold, and approximately binomial at higher voltages. Estimations of the size of contributing motor unit potentials from the variance and mean of the CMAP distributions (assuming Poisson distributions) resulted in reproducible estimates of the number of motor units contributing to the CMAP.

This may provide an alternative, indirect method of estimating numbers of motor units when direct methods are not possible, just as Poisson statistics do, in estimating quantal numbers at the end plate potential of the neuromuscular junction.

384.1

DEVELOPMENT OF LIMB TRAJECTORY FORMATION. D. L. Claman and J.H. Abbs. Dept. of Neurology & Waisman Center, University of Wisconsin 53706.

Although previous studies of reaching movements indicate invariances in spatial and temporal trajectory properties that are strongly influenced by visual information, little is known about the development of these properties.

Visually elicited planar arm movements were studied in 5-14 year old children. Movements were recorded by sliding a stylus along the surface of a digitizing table to various LED targets. In one experimental condition the target remained lighted for the duration of the trial and in the other the target extinguished at movement onset.

Analyses revealed that the nonlinear relationship between movement speed and size observed in adults for this task emerges slowly during the first 10 years of life. While movement size and velocity in adults is well fitted by a second order relation (R^2 = 0.5 to 0.94), the youngest children exhibited a much weaker coupling of velocity and extent, increasing to adult levels by age 10 to 14.

While movements executed to lighted targets yield faster movements in adults, movement time was unaffected by this additional information for the youngest children and clearly slowed movement for children in the 11-12 age range. Also, end-point accuracy, that was highly variable under both visual guidance conditions for the youngest children, improved with age; the oldest children showed both the adult pattern and the adult degree of end-point control.

The process of trajectory formation and the use of visual information in movement both appear to develop in a slow and orderly fashion through the ages 10-14, at which point more adult-like patterns are seen. Developing adult abilities to use visual information and to scale movement size and speed may underlie the performance of many motor and perceptual tasks. Supported by NIH grants (NS-13274 & HD-03352).

384.2

CONTRIBUTION OF THE EYE MOVEMENT TO THE GUIDANCE OF THE ARM

V. Delreux*, M. Crommelinck* and A. Roucoux. Lab. of Neurophysiology, Univ. of Louvain Sch. of Med., B-1200 Brussels, Belgium. (SPON: M. Meulders)

The contribution of the eye movement to the guidance of the arm not only depends on a pure visual information, but also on extraretinal factors (Mather, J. and Fisk, J., Quat. J. Exp. Psychol., 37A:315-318, 1985). A study of these factors was conducted on right handed, head fixed human subjects sitting in front of a set of LED's aligned in the horizontal plane, in the left hemifield. They were asked to move a handle, with the right hand, in the direction of one of the LED's, without visual feedback about the arm movement. In one series of experiments, the subjects kept their eyes centered; in another, they oriented their eyes, together with their arm. The target was either continually on (visual condition) or turned off three seconds before the onset of the movement (non-visual condition).

The relation of the arm final position error with eccentricity was different in eye fixed and eye mobile conditions.The characteristics of the arm error distribution were comparable in visual and non-visual conditions. The fact that eye movement, in these conditions, does not globally improve the arm precision though it modifies its characteristics, suggests that the extraretinal factors reflect mechanisms different from a simple substitution of vision.

384.3

THE ROLE OF COMPLIANCE IN CONSTRAINED BALLISTIC ARM TRAJECTORY FORMATION. D.J. Bennet and J.M. Hollerbach. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Previously we have argued that a joint level kinematic planning strategy adequately accounts for human ballistic arm movement data. In particular, we consistently observed curved trajectories when a movement between two targets spanned a joint reversal point. In the present study we pose the following question: how is a movement between such target pairs planned when it is constrained (by a frictionless wall) to be a straight line? We hypothesize that the trajectory is commanded as though the constraining wall were not there, and the natural compliance of the arm is relied upon to follow the constraint surface. This hypothesis predicts that the stiffness component of the compliance should produce an envelope of forces on the wall that resembles the unconstrained hand path. Further, we present an analysis of the time scaling property of dynamics which indicates that the differences betweeen forces on the wall in the forward (upward) and reversed directions of movement can only be due to the viscous (passive and active velocity feedback) component of the compliance. In fact, together with our hypothesis, reasonable assumptions about the arm viscosity allow the prediction that the upward and downward forces on the wall should only be equal at the elbow joint reversal point. All of these predictions are found to agree with the data. In addition, simulations qualitatively produce the experimental force envelopes.

384.4

EFFECTS OF COLD PRESSOR PAIN ON LIMB TRAJECTORY FORMATION. E.W. Howland*, D. Claman, T.A. Zeffiro, M. Backonja* AND C.S. Cleeland*. Pain Research Group and Motor Control Lab, Dept. of Neurology, Univ. of Wisconsin, Madison, Wi. 53792. (SPON: S. Zhang)

Although a growing body of research examines the effects of task performance on pain perception little is known about the effects of pain on motor performance. We studied visually elicited planar arm movements of adult volunteers by recording visually elicited hand movements made by sliding a stylus along the surface of a digitizing tablet to various LED targets. In order to reduce the effects of visual control systems, the experimental room was blacked out and the LED targets were turned off before subjects completed their arm movements.

Preliminary analysis of three subjects, each analyzed as a separate experiment, suggest that painful stimulation of the foot (0^{o} ice water) leads subjects to overshoot the target. Although overestimation of targets is typical of normal adults, pain significantly increased our subject's overshoot compared with their pain free performance. Additionally, the point of peak velocity as a fraction of total movement time decreased under pain challenge.

384.5

WITHDRAWN

384.6

EFFECTS OF AGONIST MUSCLE LOAD ON MOST-RAPID ISOMETRIC FORCE PULSES. E. Logigian*, M. Wierzbicka*, D. Cros* and B. Shahani. Massachusetts General Hospital and New England Medical Center, Boston, MA.

Contraction time (CT) of most-rapid isometric force pulses in intrinsic hand muscles has been reported to be independent of agonist muscle load (AL) prior to contraction (Freund and Budingen, 1978). We investigated the dependence of CT and EMG parameters on AL in most-rapid contractions of elbow flexor muscles to determine if the CNS motor program subserving most-rapid force pulse generation changes according to AL. With visual feedback of the force signal, elbow flexors were isometrically loaded in 5 normal men, ages 25-38, to 0,20,40,60 or 80% of their maximum voluntary force (MVF). At each AL, 10 most-rapid isometric force pulses were made to the same target: 20% MVF. In 4 out of 5 subjects, mean CT and biceps surface EMG burst duration (BD) were relatively constant only for ALs <60% MVF. At ALs >60% MVF, CT and BD significantly increased. In all subjects, the integrated biceps EMG burst (above the level to maintain the load) increased with AL, whereas triceps EMG burst area was unchanged. To generate most-rapid elbow flexor contractions of given amplitude under lower ALs, the CNS scales agonist EMG activity proportional to the load presumably by modulating motorneuron (MN) firing rate and possibly the number of MNs recruited. At highest loads, duration of MN discharge is scaled to the load, perhaps because the limits of MN firing rate and recruitment mechanisms have been reached.

384.7

CHARACTERISTIC PATTERNS OF RAPID POSITIONING ACCURACY FOLLOWING MAXIMAL ISOMETRIC CONTRACTION. B.R. Etnyre, O. Dry*. Human Performance and Health Sciences Dept. Rice University, Houston, Texas 77251

The purpose of this study was to compare post-contraction positioning accuracy and electromyographic (EMG) patterns of biceps and triceps brachii muscles during rapid elbow flexion and extension movements to a target. Following a learning session 10 subjects performed twenty control trials without augmented feedback. Experimental trials were identical to control trials except the subject preceded each movement with a 3 s isometric contraction of the agonist muscle used for the subsequent movement. Separate analyses of variance revealed significant undershooting in positioning accuracy for both flexion and extension post-contraction movements compared with control conditions. Integrated EMG (IEMG) of the agonist muscle was significantly less in the experimental condition than in the control condition for both tasks, with no difference between antagonist IEMG. Frequency Analysis of the EMG revealed significantly higher peak frequencies in the biceps during the post-contraction flexion movement. It was concluded the undershooting following isometric contraction was dependent on the total motor output of the agonist muscle for extension and an interaction of motor output and de-syncronization for flexion movements.

384.8

REFLEXES AND THE EQUILIBRIUM POINT MODEL FOR MOTOR CONTROL. J. McIntyre* (SPON: C. G. Atkeson). Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

The equilibrium point hypothesis, based on the elastic properties of muscles, is an attractive theory for the control of limb movements, but the role of reflexes in the context of this model is unclear. A hybrid control scheme is proposed which combines the equilibrium point model with reflex feedback. Computer simulation techniques are used to demonstrate the competence of this model.

In the equilibrium point model, the neuronal inputs to the muscles serve to specify an equilibrium position for the limb which smoothly varies along the desired trajectory. No explicit computation of joint torques is performed. To move faster along the same path, it is necessary to do one of two things. The stiffness of the muscles can be increased through co-contraction while the equilibrium point trajectory simply scales with movement duration. This is inefficient, as co-contraction consumes more metabolic energy per unit of time. The second possibility is that the equilibrium point trajectory is modified so that it is no longer equivalent to the desired trajectory. Instead, it may lead the actual desired position, even to the point of overshooting the desired endpoint. Computing this overshoot is essentially equivalent to computing the inverse dynamics for the system, thus losing the advantage of computational simplicity.

The proposed hybrid controller retains the computational simplicity of the equilibrium point model. The CNS needs to specify only the actual desired trajectory for the limb. The trajectory is fed directly to the muscles, as in the equilibrium point control scheme. This *virtual* trajectory is modified, however, by reflex feedback during the execution of the movement. The reflex feedback implements a PD controller which serves to increase the responsiveness of the system. This allows the system to produce faster movements without unnecessary co-contraction and without explicit dynamics calculations. Acknowledgements: Dr. E. Bizzi, Whitaker Health Sciences Fund, NIH Grant NS09343.

384.9

A CONNECTIONIST NETWORK THAT COMPUTES LIMB POSITION IN A HEAD-CENTERED COORDINATE FRAME. S.J. Hanson* and C.R. Olson (SPON: R. Cholewiak). Bellcore and Psychology Dept., Princeton NJ 08544.

Humans are able without conscious effort to turn their eyes towards a skin point stimulated in the dark. To carry out this act requires combining information about the skin location of the stimulated point with information about the angles of the joints interposed between it and the head. When many joints, each with several degrees of rotational freedom, are involved, the underlying computations are extremely complex. We have investigated the ability of a massively parallel network to carry out these computations.

The network consists of an input layer (10 units), a hidden layer (variable number of units) and an output layer (3 units). The 10 input units encode the angles of rotation about 10 axes at joints interposed between the fingertip and the head. The three output units encode the vertical and horizontal angles of the eye and its fixation distance. By use of back-propagation, the network was trained to bring the eye to bear on the fingertip given an arbitrary posture of the arm and neck.

Several runs with training sets of different sizes (100, 1000, 10000) and random starting points were carried out. In all cases, performance on the training set became extremely accurate. Performance on a transfer set improved as the training-set size increased. The properties of hidden units were characterized by use of several multivariate approaches including cluster analysis and were analyzed qualitatively by examination of Chernoff-face and barplot ("Hinton diagram") displays. The multijoint receptive fields of units in the network form a basis for predictions regarding the properties of neurons responsible for encoding body position in brain regions including area 5.

384.10

TWO PARAMETERS DETERMINE WHICH MUSCLES INITIATE PLANAR, TWO-JOINT ARM MOVEMENTS. G.M. Karst and Z. Hasan, Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724

In attempting to understand the strategy underlying CNS control of human arm movements, we have characterized movements in terms of which muscles (flexors vs. extensors at each joint) are **initially** activated during horizontal-plane pointing movements involving the shoulder and elbow. Since the observed initial muscle activity at each joint is not related simply to the direction of rotation at that joint alone, we examined the dependence of the observed initial activity on the parameters describing the initial and final limb positions. Although four parameters (e.g. initial and final angle of both joints) are required to specify these positions, we found that only two parameters are sufficient for determining initial muscle activity at each joint. This was demonstrated by a partitioning of flexor- and extensor-initiated movement trials when plotted on a plane whose axes correspond to those two parameters, namely initial elbow angle and target angle with respect to the forearm. This partitioning has proven to be a robust finding across subjects for a variety of initial limb configurations and target directions and does not appear to be affected by changes in movement velocity or by inertial loading (1.8 kg at the hand). These findings are consistent with the possibility that the CNS may use a simple criterion for determining which muscles are activated initially, while other mechanisms, including feedback, may be employed to compensate for complex dynamic effects.
(Supported by NIH grants R01NS19407 and T32NS07309.)

384.11

KINEMATICS OF TWO AND THREE LINK SAGITTAL ARM AND ARM WITH POINTER MOVEMENTS. J.R. Flanagan* and D.J. Ostry* (SPON: F. Wilkinson). McGill University, Montreal, Canada.

In an effort to understand the control of multi-joint movements, we examined two and three link human arm and arm with pointer movements between targets located in a sagittal plane. Movement path, rate, and direction as well as the inertial properties of the pointer (mass and principal moment of inertia) were varied.

The curvature of endpoint paths was found to vary with target location. Paths for three link movements were similar across differences in movement rate, direction, and the inertial properties of the pointer. Paths for two link movements were qualitatively similiar to comparable three link movements and were likewise independent of rate and direction. In general, the form of the tangential velocity curve of the endpoint scaled with rate and pointer mass and moment but not with movement direction. In many cases, the curve was positively skewed regardless of direction.

For three link movements, path and trajectory invariance of the endpoint are not sufficient conditions for joint path and trajectory scaling. In some instances, invariance in endpoint space was found in the absence of joint space invariance.

The ways in which redundancy is constrained in three link movements and the dynamics of both two and three link movements will be discussed.

384.12

THE INFLUENCE OF TASK AND ORGANISMIC CONSTRAINTS ON INTRALIMB KINEMATICS IN A DRAWING TASK. R. E. A. van Emmerik *, and K. M. Newell. Department of Kinesiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Intra-joint and joint-stylus kinematic couplings were examined as a function of various task and organismic constraints in a circle drawing task. The constraints manipulated included natural limb dominance (left-versus right-handers), the use of the dominant or nondominant limb, writing orientation (horizontal versus vertical), and circle diameter. No differences in the style of limb organization were observed between left- and right-handers (N=6), although there was a higher degree of phase-locking between the joints in the nondominant limb for right-handed subjects in the horizontal orientation. This suggests that without the constraint of writing direction, left- and right-handers adopt similar coordination functions. Correlations between the linkages significantly increased with the scaling of circle diameter, and with changing the drawing orientation from horizontal to vertical. The topological features of the stylus kinematics remained invariant under the constraints imposed, inspite of the systematic changes that occurred in the topological features of the joint motions. It is proposed that invariances and transitions in joint and stylus kinematics are a function of the task constraints that need to be optimized.

384.13

EFFECTS OF REPETITION ON ARM TRAJECTORIES DIRECTED TOWARDS MOVING TARGETS. S.I. Tillery* and J.R. Bloedel (SPON: T. Tarby). Division of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

Although many findings have been reported regarding directed limb movements in humans, few studies address the interception of moving targets. This study describes the strategies used to intercept a moving target and assesses modifications of performance occurring with practice. Subjects were instructed to maintain contact with an origin switch until a light signalled they could initiate movement towards a target moving along a 1 m horizontal track. The target velocity, ranging from 60 to 95 cm/sec, was unknown to the subject until the first trial. Three twenty trial sets, each at a different velocity, were performed for right to left and left to right target movements. Movement trajectories and EMG's for biceps, triceps, and brachioradialis were determined, and reaction time and movement duration for each trial were calculated. In general the spatial characteristics of the trajectory were quite subject specific and varied little over successive trials. Trajectories usually consisted of at least three components: an initiation phase marked by a movement towards and then away from the target's path; a high acceleration phase which brought the distal limb up to the speed of the target; and a final phase which was directed towards the target. During each phase the hand moved along a curved trajectory. The primary modification with practice was an increased acceleration during the second component of the movement. The EMG data revealed that the recorded muscles were not involved in the first phase of the movement. The data indicate that the trajectory's envelope is subject specific and that changes in performance associated with practice do not alter the spatial characteristics of the movement. Supported by NIH grant NS21958.

384.14

CONTROL OF SINGLE- AND MULTI-JOINT MUSCLES DURING THE MAINTENANCE OF A POSTURE AT THE HUMAN ELBOW JOINT. T.S. Buchanan, G.P. Rovai*, and W.Z. Rymer. Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611.

We examined the activation of muscles that act in elbow flexion/extension and wrist supination/pronation during various static postures. Intramuscular and surface EMGs for five muscles and force at the wrist in three dimensions (flexion/extension, varus/valgus, and supination/pronation) have been simultaneously recorded while subjects produce proscribed steady-state forces at the wrist. Tests were done with subjects at three different degrees of forearm supination/pronation.

We found that some elbow-joint muscles that do not contribute to supination/pronation torque generation are nonetheless varied significantly during these loads. For example, when the wrist is in a supine position, the triceps acts as if it were a supinator—EMGs increase with increasing supination load, all other load conditions being the same. However, when the wrist is held in a pronated position, the triceps mimics a pronator, increasing its EMG activity with increasing pronation torque. Other muscles were also significantly influenced by supination/pronation position. Biceps and pronator teres varied as might be expected, but variations in brachialis were unforeseen.

Since supination/pronation and flexion/extension are performed at independent orthogonal joints, it is surprising to see some muscles at one joint being modulated with loads at another where they have no obvious mechanical action. This is certainly the case for triceps and brachialis. Such observations point to a complex control strategy that provides neural coupling of muscles at nearby joints even when biomechanical coupling may not exist. Such coupling is perhaps important for counteracting inappropriate torques produced by multi-joint muscles that are necessary for torque production. For example, the triceps may be acting to offset torques produced by the biceps which generates elbow flexion torques as it aids in supination.

This work is supported by NIH grant NS-19331.

384.15

PATTERNS OF ACTIVITY IN WRIST EXTENSORS DEPEND ON FOREARM POSITION. D.S. HOFFMAN and P.L. STRICK. VA Med. Ctr. and Depts. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse, NY 13210.

Monkeys were trained to perform wrist movements in 8 different directions. The task was performed with the forearm in 2 positions: fully pronated or midway between pronated and supinated. We examined the patterns of activity in wrist extensors using pairs of fine wire electrodes. The electrodes also were stimulated to determine each muscle's direction of action.

When the forearm position was changed from pronated to the mid position, the direction of maximal agonist activity in all wrist extensors shifted 20-100 degrees in the radial direction. The shift in forearm position also altered the direction of movement produced by electrical stimulation for some, but not all wrist extensors. For example, when the forearm was pronated, agonist activity in extensor carpi ulnaris (ECU) was largest for ulnar deviation. When the forearm was in the mid position, agonist activity in ECU was largest for wrist extension. This shift occurred even though ulnar deviation was evoked by electrical stimulation of ECU in either forearm position.

These results indicate that the activity of a wrist muscle can be dissociated from: the muscle's direction of action, the direction of joint movement and the direction of movement in space. Supported by funds from the VA Medical Research Service.

384.16

RAPID REPROGRAMMING OF MUSCLE ACTIVITY DURING VOLUNTARY MOVEMENT. Jerome N. Sanes, NINCDS, NIH, Bethesda, MD 20892.

When voluntary movements are mechanically disturbed, short-latency muscle responses are triggered. The latencies of these responses are similar to responses evoked during postural maintenance. There is a short-latency, presumably myotatic, and then longer-latency responses that depend on mechanical conditions, instructions and the input stimulus. The present experiment investigated whether these triggered muscle responses were similar to responses observed when subjects performed unobstructed movements that started from the obstruction location.

Subjects performed 30° wrist flexion movements in 200±20 msec by moving a handle attached to a torque motor while the surface EMG was recorded from the wrist flexor and extensor muscles. One series of movements began at 0° and terminated at 30° of flexion. A second series of movements started at 10° of extension, 0° or 10° of flexion. In separate series of 100 trials each, 25% of the movements were stopped for 100 msec after 5°, 15° or 25° of movement. The time to complete the movement and the peak velocity after offset of the stop were measured. Subjects then performed movements that were regulated according to the measured movement times (MT) or peak velocities (PV). The triphasic bursts in the EMG were quantified during voluntary movement and after the stop onset according to conventional techniques.

Triggered muscles responses did not always resemble muscle responses accompanying unimpeded voluntary movements beganning from the angular location of the stop. The first agonist burst (AG1) triggered by a stop encountered 5° and 15° into the movement was smaller than the AG1 during unobstructed movements. The antagonist and the second agonist bursts triggered by the stop were typically similar to the EMG observed during unobstructed movements. There were not major differences in response characteristics when subjects regulated MT or PV.

These results illustrate that muscle responses triggered during active movements do not always resemble the EMG of unobstructed movements. The triggered muscle responses that differ from EMG patterns of unimpeded movements are those with short latency but only if they are triggered early in the movement. These results suggest that there is a temporal limit in which triggered EMG responses can be modified to compensate for perturbations occurring during movement. When this temporal limit is exceeded triggered EMG responses resemble programmed responses suggesting that these later responses do not contribute to compensation.

384.17

UNILATERAL, BILATERAL AND INTERLIMB COORDINATION OF REPETITIVE MOVEMENTS. R.A. States, C.C. Bassile*, A. Dettwiler-Danspeckgruber*, J.A. Monroe*, A.M. Gentile. Teachers College, Columbia University; NY,NY 10027.

This study investigated coordination of repetitive movements involving one, two or four limbs. Tasks required tapping movements of the hand or foot in the sagittal plane performed at self-determined preferred and fast rates. As prior work (Kay et al., J. Exp. Psych.: HP&P, 1987, 13:178-192) has shown greater stability for simultaneous (0° phase diff.) over alternating (180° phase diff.) movements in bimanual tasks, these two coordinative modes were used in the present study for bilateral (2 limb) and interlimb (4 limb) tasks. Data collected on 8 seated subjects included movement kinematics (period, amplitude, velocity) and for bilateral and interlimb tasks, measures of phase difference and dispersion. At preferred rates, tapping period and variability (coefficient of variation) were comparable for unilateral and bilateral tasks in the simultaneous mode; whereas, in the alternating mode, periods were longer and less variable. No differences between hands and feet were noted for those tasks. In interlimb tasks, similar differences in tapping period and variability were observed between simultaneous and alternating modes. While tapping period did not differ between limbs; the feet were significantly less variable than the hands under the alternating mode. For both bilateral and interlimb tasks, alternating movements were more strongly coupled (as measured by phase dispersion) than were simultaneous movements, especially for the feet. Thus, the major finding is that in interlimb coordination, alternating movements of the feet are more consistent and more strongly coupled than the hands. This coupling may utilize an endogenous neural synergy associated with locomotion as suggested by A.M. Gentile & R.A. States (Neurosci. Abs., 1987, 2:321.10).

384.18

THREE DIMENSIONAL TRAJECTORY ANALYSIS OF A PATIENT WITH CONGENITAL MIRROR MOVEMENTS. H. Poizner and M.Kritchevsky*. The Salk Institute, La Jolla, CA 92138, Department of Neurosciences, UCSD, La Jolla and V.A. Medical Center, San Diego.

Mirror movements are involuntary movements executed by one side of the body in response to voluntary activation of homologous muscles of the other side. Although such movements have been described qualitatively and with surface EMG recordings, the spatial and temporal characteristics of these movements remain relatively unexplored. Selected simple, repetitive and complex limb movements were studied in a 20 year old woman with congenital mirror movements and no other neurological disorder. Movements were digitized in three dimensional space, reconstructed computergraphically, and analyzed numerically and graphically. Mirror movements had smaller amplitudes than did the corresponding voluntary movements and there was, in general, temporal coupling between mirror and voluntary movements. Nonetheless, mirror movements were not always a perfect mirror image of the corresponding voluntary movements and sometimes differed in timing and trajectory shape from the original movement. Substantially greater mirror movements were elicited by distal as opposed to proximal movements and mirror movements were enhanced when loads were applied to the hand executing the voluntary movement. These data will be related to anatomical bases of congenital mirror movements.

384.19

COORDINATION DURING A MULTI-JOINT ARM MOVEMENT IN NORMAL HUMANS AND IN PATIENTS WITH CEREBELLAR DYSFUNCTION. W.J.Becker,E.Kunesch*,H.-J.Freund. Neurologische Klinik, Universität Düsseldorf,FRG.

Coordination was studied during a multi-joint arm movement in 3 controls and in 4 patients with cerebellar degenerative diseases and moderate ataxia.

While subjects threw a ball at a target, EMG activity was recorded from 7 arm muscles(Glonner EMG telemetry system) and position data was recorded from 6 points on the arm (Selspot II system). In this movement, triceps(T) and wrist flexors(WF) act synergistically at different joints to accelerate the hand. 31 single movement trials were analyzed for each group. Even though patients threw much less accurately than controls, the mean time interval (T-PV) from T EMG onset to time of distal forearm peak velocity, an indicator of T function, was similar in controls (181+18) and patients (183+18ms). The time interval by which T EMG onset preceded WF EMG onset(T-WF) was shorter in movements with a shorter T-PV interval,allowing for WF to assist in hand acceleration prior to ball release. This correlation between T-WF and T-PV was present in controls (r=0.80) and patients (r=0.70).

The aspects of coordination assessed in this study may be mediated by CNS structures outside the cerebellum. Alternatively, the normal cerebellum may have sufficient functional reserve to allow it to participate in these coordination mechanisms despite marked atrophy and cell loss.

384.20

IMPAIRMENT IN PROGRAMMING OF RESPONSE DIRECTION AND AMPLITUDE IN DEAFFERENTED PATIENTS. C. Ghez, R Bermejo, & J. Gordon. Ctr for Neurobiol & Behav, Columbia Univ and NYS Psych Inst, New York, NY 10032.

We have previously shown that the accuracy of phasic isometric responses is markedly degraded in patients with severe large fiber sensory neuropathy. Inaccuracy derived from impairment in the implementation of the normal pulse height control and an inability to correct initial trajectory errors by compensatory variations in rise time. The present experiments were done to determine if the control of hand trajectories in two-dimensional space is also degraded.

Patients and normal controls were to move a hand-held cursor from one target to another on a digitizing tablet. Cursor and target positions were displayed on the screen of a Macintosh computer and vision of the hand was prevented. Several different tasks were used to assess the accuracy of fast and slow movements with visual guidance (screen cursor visible) and without visual guidance (screen cursor blanked). In addition, visuomotor learning was assessed by examining the time required to adjust the direction of visually guided movements when the tablet was rotated 45°. In deafferented patients, rapid visually guided movements from one target to another could be performed accurately only by fragmentation of the trajectory into smaller segments with lower than normal peak velocities. When vision was prevented, patients showed errors in amplitude and direction that were markedly greater than those of normals. Whereas, in the absence of visual feedback, normals could reproduce a given trajectory several times without substantial changes, in patients, the accuracy of sequential movements was further degraded with each repetition. In contrast, one patient thus far tested did not show any specific impairment in the ability to adapt to rotations of the relationship between the spatial coordinates of the hand and of the screen cursor. We conclude that somatosensory information from the limb is necessary to accurately specify the direction and amplitude of responses aimed to visual targets, but may not be necessary to learn a visuomotor transformation. Accurate programming apparently requires information concerning the initial state and position of the limb. (Supported by NS22715)

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: POSTURE AND MOVEMENT VII

385.1

CHANGES IN MOVEMENT KINEMATICS DUE TO CEREBELLAR DYSFUNCTION. S.H.Brown, H.Hefter*, M.Mertens*, J.D.Cooke & H.-J.Freund. Dept. of Neurology, Univ Düsseldorf, FRG & Dept. of Physiology, Univ. Western Ontario, London, Can.

The role of the cerebellum in the generation of goal-directed, single joint movements was studied in 10 patients with mild cerebellar deficits. Step-tracking elbow movements were performed at 4 amplitudes and under 3 instructions.

Peak velocity (PV) and movement duration (MD) increased linearly with amplitude. Mean PV and MD values showed slight differences between cerebellar and control groups. Variability of PV and MD, however, was greater in the cerebellar group, especially during slow movements. Marked differences in the relative durations of acceleration and deceleration (symmetry) of the velocity profile occurred across all movement conditions. In all patients, both continuous and discontinuous velocity profiles showed prolonged decelerations. The degree and variability of movement asymmetry increased as the demand for accuracy increased.

These findings support the view that movement trajectory, particularly the deceleratory phase, is under cerebellar control.

(Supported by a von Humboldt Fellowship to SHB)

385.2

EXPERIMENTAL-THEORETICAL ANALYSIS OF THE INTRINSIC GEOMETRY OF LIMB MOVEMENTS *J.R. Bloedel, S.J. Tillery* and A.J. Pellionisz* (Barrow Neurological Institute, Phoenix, AZ, 85013 and Dept. Physiology and Biophysics, New York Univ. Medical Center, New York, NY, 10016)

Problems in motor control are difficult to address without a quantitative model of the motor plant. To assist experimental investigation of locomotor and volitional forelimb movements in the cat, a preliminary computer model of its skeletomuscular system has been constructed and applied to an analysis of the step cycle. The approach is based on the calculation and experimental establishment of coordinate frames that are intrinsic to the skeletomuscular system. Software to calculate intrinsic coordinates from a graphic representation of joints and skeletal muscle attachments was adopted from models of head movements in cats. (*Pellionisz and Peterson*, in: Control of Head Movements, Oxford U. Press).

Specifically, the pulling directions of ten forelimb muscles were determined by first assessing the origin and insertion of each by dissection and then calculating the direction the limb moves from specific locations in the step cycle when the muscle is shortened. These pulling directions were checked qualitatively by observing the forelimb movement evoked by tetanically stimulating each muscle in isolation. Next these directions were used to establish the axes of an intrinsic coordinate system on which the model was based. The most striking finding was that the spatial features of the trajectories could be predicted based on the intrinsic coordinates and the direction of the intention vectors, revealing the functional geometry underlying the execution of the locomotor cycle. Predicted and observed trajectories were compared to determine the extent to which the metric of the intrinsic coordinate system had to be updated to yield a movement whose trajectory was similar to that found experimentally. This updating required the recalculation of the Eigenvectors and the components of the metric tensor during movements. The application of this approach resulted in the generation of "curved geodesics" or trajectories which approximated the locomotor behavior of the animal. These data provide a rationale for further pursuing the basis for the functional geometry underlying the spatial properties of limb movements. *Supported by NIH grants NS22999 and NS21958.*

385.3

IBOTENIC ACID LESIONS OF THE RAT RED NUCLEUS. J. Tomie,* R.L. Ladowsky and I.Q. Whishaw, (SPON: E. Castaneda) University of Lethbridge, Alberta, Canada.

Anatomical and electrophysiological evidence suggests a role of the red nucleus (RN) in limb movement, yet its importance is still not understood. The early work of Lawrence and Kuypers found that sectioning of the rubrospinal tract in monkeys produced transient deficits in the use of the ipsilateral arm, whereas combined sectioning of the pyramidal and rubrospinal tracts produced severe and lasting deficits. The present study examined paw use in rats following ibotenic acid lesions of RN.

Rats were trained to reach for food through bars separated by 9 mm, and their paw preferences were established. The RN contralateral to their preferred paw was then infused with ibotenic acid, and their non-preferred paw was bandaged to prevent its use. After recovery, the number of times the rat successfully reached for food with its preferred paw was compared to pre-operative success rates.

Histology showed complete cell loss in both the parvicellular and magnocellular divisions of RN, with minimal damage to nearby structures. Such lesions seem not to impair reaching. These results support previous notions of partially redundant pyramidal and rubrospinal tract function.

385.4

EFFECTS OF GLOBUS PALLIDUS LESIONS IN RATS ON BALLISTIC FORELIMB FORCE EMISSION. J.V. Harrell and R.C. Hicks*. Dept. of Psychology, Hampden-Sydney Coll., Hampden-Sydney, VA 23943

Rats were trained to press with their forepaws on a force transducer under a fixed-ratio 5 (FR5) schedule of reinforcement for water reinforcers (Harrell & Hayes, Neurosci. Abst., 16:1224, 1985). Four rats received bilateral, electrolytic lesions of the globus pallidus (GP) and two rats were unoperated controls.

Rats were trained on the required task until a stable baseline of 20 days data was collected. Surgeries were then performed and on following day testing under preoperative criteria continued. The initial postoperative testing period lasted 27 days. Thereafter ensued a six week hiatus followed by 57 additional testing days. A total of 100 days elapsed from performance of surgeries to the conclusion of the experiment.

Three of the four GP rats showed relatively permanent effects of the lesions. These effects, shown in strip chart recordings, were characterized by a decrease in ability to perform rapid bursts of responses and by an increase in the time between bursts. There were also noticeable changes in postural adjustment capability. While greater performance deficits were observed in this study than in a previous study of effects of caudate nucleus lesions in rats on the same task, essential ability to perform the forelimb response remained unaffected.

(Supported in part by a grant from the Gwathmey Foundation of Virginia and an H-SC student research award)

385.5

TASK DEPENDENCE OF CROSS CORRELATIONS BETWEEN MONKEY RED NUCLEUS AND FORELIMB MUSCLE EMG.

L.E. Miller, P.L.E. van Kan* and J.C. Houk Dept. Physiol., Northwestern Univ. Med. School, 303 E. Chicago Ave, Chicago, IL 60611.

In a prior study, the cross-correlation function was used to assess functional relations between unit discharge in magnocellular red nucleus (RNm) and different forelimb electromyograms (EMGs) recorded while cat subjects performed a food retrieval task (J. Neurosci. Meth. 21: 201, 1987). The goal was to define the spatial and temporal properties of linkages between RNm and limb muscles. A major problem that arises with conventional correlation methodology is the lack of objective criteria for distinguishing between causal and non-causal linkages. Here we present theoretical simulations and experimental data from monkeys suggesting that one can assess the likelihood that a linkage is causal by introducing variations in the behavioral task.

Stereotyped movements such as reaching to the same point in space are particularly susceptible to non-causal correlations because different muscles are activated in a fixed temporal sequence. Muscle activity becomes behaviorally coupled. Under these conditions, if a central neuron is causally linked with any one muscle, other muscles will also be correlated at a variety of lag or lead times. Simulations we performed using the NEXUS software package (Comput. Biol. Med., 14: 385, 1984) suggest that combining several tasks with significantly different temporal sequences of muscle activation reduces non-causal correlations toward zero, without affecting correlations in causal linkages. Experimental data supporting this conclusion was obtained from monkeys trained to perform several movement tasks, each of which evoked distinctly different EMG patterns. RNm unit discharge was recorded simultaneously with EMG activity from 8-14 forelimb muscles. We assessed the extent of behavioral coupling between muscles with cross-correlations between EMGs. When behavioral coupling was decreased, the number of muscles that remained well correlated with RNm discharge also decreased. Correlations that remain under these conditions may represent causal linkages, whereas those that disappear are likely to be non-causal.

385.6

FIELD OBSERVATIONS OF HAND MOVEMENTS AFTER DORSAL COLUMN SECTION. C. M. Leonard, C.J. Vierck, Jr., and B.Y. Cooper. Dept. Neuroscience, Box J244, JHMHC, University of Florida, Gainesville, FL 32610.

The accompanying abstract by Cooper et al. reports that monkeys (Macaca arctoides) with dorsal column lesions are permanently impaired in performing multi-articulated, fractionated finger movements when tested in an experimental paradigm. This permanent deficit contrasts with a temporary decrease in the use of the affected limb for walking, climbing, and grooming others. 3 of 4 monkeys showed grossly normal groom patterns within 3 weeks of surgery, including the ability to precisely appose finger and thumb in the precision grip. The 4th animal demonstrated apposition at 3 months. Frame by frame comparisons of pre- and post-surgery videotapes reveal occasional subtle changes in the spacing between the digits that suggest altered mechanisms of control. There is a long-lasting decrease in the use of the limb for self-scratching, foraging and manual sexual investigation. The decrease in scratching was unexpected. Scratching is a stereotyped behavior performed at a fixed rate of 5/sec and does not require either multi-articulation or individual finger movements. It had not previously been thought to depend on intact dorsal columns. Field observations combined with specialized laboratory tests can provide new insights into dorsal column function. (Supported by NS 13516 and NS 17474.)

385.7

Finger movement disorders following section of the dorsal columns. Cooper, B.Y., Schulmann, D.L., Leonard, C.M., and Vierck, C.J., Jr. Dept. of Neuroscience, Box J-244, JHMHC, University of Florida, Gainesville, Florida, 32610.

The present experiments examined the role of the dorsal column (DC) pathway in fine finger movements. A task was designed to assess the ability of subhuman primates to perform, fractionated (individual), ballistic finger movements that required complex multiarticulations (flexion-extension combinations). A second task was designed which assessed both fine adjustments and smooth pursuit of fingers to a moving tactile cue (air puff).

Following lesions of the DCs, deficits were observed on both tasks. Monkeys with DC lesions had enduring deficits on both fractionated, ballistic, and non-fractionated, pursuit, finger movement tasks. Performance on the spared side was comparable to pre-lesion levels. Deficits were characterized by: 1) loss of individual finger movements; 2) loss of complex, multiarticulated finger movements; 3) increased finger movement force; 4) excessive movement range during initiation and terminal phases of movement; 5) failure to track a moving tactile cue (fine adjustive movements) in either flexion or extension; and 6) poor hand orientation to goal objects. Field observations confirmed that monkeys could walk, climb and perform some precise grooming movements (see Leonard et al., 1988, in this volume).

Importantly, pincer movements remained functional. Others have concluded, on the basis of preserved pincer movements, that DC lesions do not impair finger movements.

385.8

FASTIGIAL, INTERPOSED, AND DENTATE NUCLEI: SOMATOTOPIC ORGANIZATION AND THE MOVEMENTS DIFFERENTIALLY CONTROLLED BY EACH. S.A. Kane, J.W. Mink, and W.T. Thach. Dept. of Anatomy, Washington Univ. Sch. Med., St. Louis, MO. 63110.

Rhesus monkeys performed 4 types of wrist movements with and against torque loads: 1) visually cued ballistic movement (VisStep), 2) somesthetically cued hold-perturbation-hold movement (PertHold), 3) slow, visually guided hold-ramp-hold ramp tracking movement (VisRamp), and 4) self paced, rapid alternating movement (RAM). VisStep was performed under 3 types of torque loads: 1) constant torque, 2) viscous, which diminishes tremor amplitude, and 3) negative-viscous, which enhances tremor amplitude.

Fastigius: neurons were not related to these wrist movements. Focal inactivation with microinjection of muscimol gave no deficit in these movements, but instead severe deficits in standing and walking, with weakness of the antigravity muscles of ipsilateral limbs, and falling to the ground on that side. Different injections produced stance- and gait- specific weakness of the arm or the leg only, with the leg represented anteriorly in the nucleus and the arm more posteriorly, as had been previously shown for interposed and dentate nuclei.

Interpositus: In VisStep, neurons changed activity near onset of movement, but inactivation did not delay movement onset. In PertHold, neurons had short latency response (13 msec) to the perturbation (also motor cortex); inactivation delayed the response in motor cortex and in the behavior. In VisRamp, neurons discharged in relation to tremor that decreased under viscous load and increased under negative-viscous load; inactivation increased physiological tremor. In RAM, neurons were phasically active; inactivation made RAM slow and irregular.

Dentate: In VisStep neurons changed activity early before movement onset; inactivation delayed movement onset. In PertHold, neurons had only long latency (20 msec) response; inactivation did not delay behavioral response. In VisRamp, neurons had no tremor signal even when the tremor was exaggerated by the negative-viscous load; inactivation did not increase the physiological tremor. In RAM, neurons were phasically active; but inactivation did not impair this movement.

These results suggest somatotopically organized, functional zones in the cerebellum: fastigius controls antigravity muscle synergies in standing and walking; interpositus controls stretch and other somatosensory reflexes and helps damp tremor; dentate helps initiate volitional movements. (Supported by Washington University's Division of Biology and Biomedical Sciences, NIH grants NS12777 and NS15070, and the McDonnell Center)

385.9

DOPAMINE ANTAGONIST ALTERS THE TRAJECTORY OF THE ELBOW JOINT BUT HAS NO EFFECT ON THE FINAL POSITION GENERATED BY MONKEYS POINTING AT VISUAL TARGETS. R.M. Wylie and A. Martinez-Arizala. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

To better understand the role of the nigro-striatal system in movement, we have studied the effect of a dopamine antagonist, metoclopramide, on an elbow flexion task performed by Rhesus monkeys. Three monkeys were trained to track a visual target by rotating their forearms about the elbow in a horizontal plane. The target moved stepwise and the monkeys were required to point to within five degrees of it. Successful responses were rewarded with a liquid nutrient. Unsuccessful trials earned no reward. We recorded acceleration, velocity and position. Effective doses (0.3 to 0.4 mg/kg i.m.) reduced the peak of acceleration and of velocity, but had no effect on final position. Were the effect on acceleration a simple scalar effect, then position, as the second integral of acceleration, should be reduced by the same scalar value. Because the final position does not change significantly, we conclude that the system compensates for the reduction in acceleration. We see evidence of compensation in the increase in the risetime of acceleration and of velocity as well as an increase in the duration of the period of positive velocity, that period during which the arm moves toward the target. We cannot resolve the issue of whether compensation arises internally or reflects the operation of a feedback control system.

385.10

REACHING TO FAR VS. NEAR CONTRALATERAL EXTRACORPOREAL HEMISPACE BY MONKEYS WITH HEMINEGLECT FROM CORTICAL LESIONS Ruthmary K. Deuel. Department of Pediatrics, Washington Univ. Sch. of Med., St. Louis, MO 63110.

In monkeys with cortical removals leading to neglect of corporeal and extracorporeal space, ipsilateral (IPSI) hand use is often impaired in contralateral (CONTRA) space. To test the latencies of reaching for a salient bit of bait near (15°) or far (70°) from visual fixation, the animal was seated in a primate chair with its head in a viewing box and the hand CONTRA to the lesion (or future lesion) restrained. When the head was voluntarily fixed forward and the IPSI hand was voluntarily resting on the chair waist piece, the view box was opened. If the eyes were fixed forward, a piece of bait appeared in one quadrant. The latency from bait presentation to breaking of a light beam by the reaching hand was measured. Preoperatively, in the 15° reach most IPSI hands were faster in the IPSI hemifield. For 70°, the IPSI hand tended to shorter latencies in the CONTRA field, with cross reaching adopted as an efficient strategy. Postoperatively animals tended to faster latencies than preoperatively in both 15° and 70° IPSI fields, contrasted with increased latencies in the CONTRA fields; however without gradation of severity from 70° to 15° CONTRA fields for the group. Individual choice of motor strategy rather than an obligatory visual neglect of far contralateral space appears to have determined these results.

385.11

COMPUTER MODELING OF HUMAN FORELIMB MUSCLE ACTIVATION IN MULTI-DIMENSIONAL INTRINSIC COORDINATE FRAMES *J. Laczkó** (Central Research Institute for Physics, Budapest, 1525 Hungary), *A.J. Pellionisz*, (Dept. Physiol. New York Univ. Med. Ctr. 10016 USA), *H. Jongen*, and *C.C.A.M. Gielen* (Dept. Med. & Physiol. Physics, State U. Utrecht, 3584 Holland) (SPON: *G. Ostriker)*

Computer modeling of motor control of body-appandages initiated with single-dimensional analysis of (paired) muscles of "limbs with a single joint"; the eye. Skeletomuscular systems with *distributed centers of rotation*, such as the head-neck system of the cat *(Pellionisz and Peterson* 1988; In: Control of Head Movements, Oxford U. Press, eds. Peterson & Richmond), required development of graphics-based software (*Laczkó et al.* 1987 Neurosci.Abst. 13:372), where origin and insertion areas of muscles are "carried" with movements of skeletal elements and the changing multi-dimensional coordinate system, intrinsic to activation of motor units, is (re)computed.

Utility of a multidimensional approach is twofold. First, quantitative interpretation of experimental data on complex appendages calls for suitable comprehensive handling (cf. *Gielen and Zuylen*, Neuroscience: 86,17:527). Second, in order to implement, in *neurocomputing and (neuro)robotics*, a theoretical understanding of how biological organisms coordinate movements, a formal expression of the underlying mathematical principles is necessary, such as coordination in Eigenvector-frames (cf. *Pellionisz*, 1984 J.Theor.Biol. 110:353). Basis of developing a model of human arm is *computerized anatomy*, quantitating the structure of an overcomplete number of skeletal elements, joints, spatial arrangement of up to 22 muscles and, ultimately, the much higher number of heterogeneous motor units and motoneurons. Problems of motor control, such as coordination and trajectory-formation can be addressed in such multidimensional intrinsic coordinate systems (cf. *Pellionisz, Soechting, Gielen, Simpson, Peterson, Georgopoulos*, 1986 Neuroscience Abst.12:1). The present preliminary model (representing 2D movements) of the human forearm is based on anatomical data, and is aimed at interpreting physiological measurements, both sets obtained in the Utrecht laboratory. A video will demonstrate the present level of the suitability of the model to geometrize CNS motor control in general non-orthogonal intrinsic coordinate frames and their underlying characteristic Eigenvector-systems. *Supported by NS-22999*

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM III

386.1

RESPONSES OF ABDUCENS NUCLEUS NEURONS TO VESTIBULAR STIMULATION IN AWAKE RABBIT. J.S. Stahl and J.I. Simpson. Dept. Physiol. & Biophys., NYU Med. Ctr. NY, NY 10016

Single abducens nucleus neurons were recorded extracellularly in awake rabbits and antidromically identified as motoneurons (MN) or internuclear neurons (INT). Firing rate and eye position were obtained during sinusoidal yaw rotation in light and dark (.05-0.4 Hz, 3 °/sec peak velocity). The majority of MN went into cutoff for a substantial part of the stimulus cycle, suggesting that in the rabbit, recruitment occurs at more abducted eye positions than in cat and monkey. The neural response was analyzed using linear multivariate regression to yield measurements of eye position sensitivity (k) and eye velocity sensitivity (r). For MN at 0.1 Hz in the light, the average values were 16.6 spikes/sec/° and 10.3 spikes/sec/°/sec for k and r, respectively (n=5). For INT the corresponding values were 12.0 and 8.9 (n=8). These k and r values are higher than those of the cat and monkey. For both MN and INT, k increased and r decreased with stimulus frequency. For MN, the ratio r/k (T) decreased from an average of 1.00 sec at .05 Hz to 0.45 sec at 0.2 Hz. For INT, the corresponding decrease was from 1.09 to 0.49 sec. The frequency dependence of T means that the oft-used first-order model relating firing rate and eye kinematics is incomplete even for the slow phase of vestibular nystagmus. Supported by NS-13742.

386.2

TROCHLEAR MOTONEURONS DECREASE THEIR FIRING RATE FOR OCULAR CONVERGENCE.
L. E. Mays and P. D. R. Gamlin. Department of Physiological Optics, School of Optometry, University of Alabama at Birmingham, Birmingham, AL 35294.

It has long been known that the relationship between ocular torsion and eye position is altered with convergence. Allen and Carter (Am. J. Optom. 44:343, 1967) have shown that most subjects exhibit a small degree of excyclorotation for convergence eye movements. We have examined the firing patterns of 13 trochlear (superior oblique) motoneurons in two Rhesus monkeys trained to make symmetrical vergence eye movements. The horizontal and vertical positions of both eyes was measured using search coils. Ocular torsion was not monitored. Since the secondary action of the superior oblique is depression, a strong relationship between vertical eye position and firing rate was observed (3.86 spikes/sec per degree). The relationship between firing rate and horizontal conjugate gaze shifts was weak. For convergence, however, a clear decrease in firing rate was seen in all cells tested. This decrease (1.57 spikes/sec per degree of vergence adduction) was 5 times larger than that seen for similar conjugate adduction. This decrease, and consequent relaxation of the superior oblique muscles, appears to be responsible for the excyclorotation seen during convergence. (Supported by NIH Grant EY03463 and P30 EY03039).

386.3

RESPONSE PROPERTIES OF NEURONS IN THE PRETECTAL NUCLEUS OF THE OPTIC TRACT OF BEHAVING PRIMATES. M.J.Mustari and A.F.Fuchs. Regional Primate Research Ctr., Univ. of Washington, Seattle, WA 98195.

The pretectal nucleus of the optic tract (NOT) is thought to play a role in the horizontal optokinetic reflex in many lower mammals. To examine its role in primates we have studied the discharge properties of 81 pretectal units that were histologically verified to be in the NOT of the rhesus monkey.

The visual properties of NOT units were tested with large-field random dot stimuli that moved while the monkey fixated a stationary target spot. NOT units were sensitive to both the velocity and direction of the visual stimulus. Their response generally was strongest for stimuli that had an ipsilaterally directed component. Unit firing rate was monotonically related to stimulus velocity over at least part of the range (0-200 deg/sec) tested. In addition to responding to large-field stimuli, 27% of NOT units also discharged during smooth pursuit of a small target spot. If the target spot was extinguished briefly during pursuit, but the monkey continued tracking, the responses of the majority (90%) of such units dropped to their resting rates, indicating that they were responding to the visual slip of the target during imperfect tracking. Finally, weak electrical stimulation at the sites of NOT units elicited optokinetic-like eye movements with time courses similar to those generated by optokinetic drums. We conclude that the NOT could play a role in smooth pursuit or optokinetic eye movements.

386.4

MEDIUM— LEAD BURST NEURON ACTIVITY DURING OBLIQUE SACCADES. J. S. Nelson*, R. Hartwich-Young and D. L. Sparks. Neurobiology Research Center and Department of Physiology & Biophysics, University of Alabama at Birmingham. Birmingham, Alabama 35294.

The activity of medium-lead burst (MLB) neurons in the paramedian pontine reticular formation (PPRF) has not been studied extensively during oblique saccades. Nevertheless, recent models of the saccadic system make explicit assumptions about the behavior of these cells during oblique saccades (Grossman & Robinson, 1988; Scudder, 1984; Tweed & Vilis, 1985; van Gisbergen et al., 1985). We have generated a body of empirical data that can be used for the evaluation and development of these models. Four rhesus monkeys were prepared for chronic recording and measurement of eye movements using the scleral search coil technique. The monkeys were trained on a saccade task requiring acquisition of a fixation target and an eccentric target. The targets were an array of LED's on a tangent screen. Insulated tungsten electrodes were placed in the PPRF close to the abducens nucleus. Burst neurons were isolated, and their saccade-related activity was recorded for saccades having a wide range of directions and amplitudes. Although the velocity of the horizontal component of oblique saccades and saccade direction are highly correlated, our results indicate that the relationship between burst frequency and saccade direction is stronger than the relationship between burst frequency and the velocity of the horizontal component. First, burst frequency remains relatively constant for saccades of the same direction that differ in vectorial amplitude and, therefore, display a large range of horizontal amplitudes and velocities. Second, the curvilinear relationship between burst frequency and saccade direction is often asymmetric and different in shape from the relationship between saccade direction and the peak velocity of the horizontal component. These findings indicate that the MLB frequency/velocity relationship is direction specific.(Supported by NIH EY01189 and EY05914).

386.5

ACTIVITY AND PONTO-BULBAR CONNECTIONS OF RETICULO-SPINAL NEURONS SUBSERVING VISUALLY TRIGGERED ORIENTING EYE AND HEAD MOVEMENTS. A. Grantyn+, O. Hardy+ and A. Berthoz.
Lab. de Physiologie Neurosensorielle, CNRS, Paris, France.

Descending axons in the caudal pons or rostral medulla were recorded intracellularly in alert, head-fixed cats performing orienting to moving visual stimuli. Spike activity was examined for correlations with eye movements and attempted head movements (EMG of dorsal neck muscles). Neurons related to these motor events and receiving mono- or disynaptic excitation from the contralateral superior colliculus were injected with HRP and their axonal trees reconstructed. The sample includes 11 reticulo-spinal neurons (RSN) projecting in RSTm. The types of their signals form a continuum which can be illustrated by 3 representative groups: 1) Phasic RSN generating short bursts linked to the onset of saccades and/or associated neck muscle activity; 2) RSN eventually showing prolonged bursts, frequency modulated in relation to saccades and early parts of dynamic EMG-components; 3) RSN with modulation of spike rate reflecting the profiles of even the longest EMG-transients and nearly lacking saccade-related components. HRP labeling reveales that RSN of groups 1, 3 contact the reticular core only, by a few (1-5) poorly branched collaterals. Group 2 RSN show multiple (8-15 collaterals) branching in the ponto-bulbar RF, prepositus/vestibular complex, and abducens and facial nuclei. The data indicate a distributed nature of the premotor signal in the tecto-reticular network and disclose some aspects of its intrinsic connectivity.

386.6

ROSTRAL OUTPUT NEURONS OF SUPERIOR COLLICULUS ARE ACTIVE DURING ATTENTIVE FIXATION. D.P. Munoz and D. Guitton.
Neurology and Neurosurgery, McGill Univ., Montreal, Canada

The superior colliculus (SC) plays an important role in controlling orienting behaviour. A major projection neuron emanating from the deeper layers of the cat SC, the tecto-reticulo-spinal neuron (TRSN), collateralizes in regions of the brainstem and spinal cord involved in controlling eye and head movements. The rostral SC contains an extensive representation of the central visual field. Here we report the discharge characteristics of TRSNs located in this region. Antidromically identified TRSNs were recorded extracellularly in head-fixed/free cats trained to perform several visuo-motor tasks (Brain Res. 398:185). Results: 1)Rostral TRSNs responded to visual, auditory, and somatosensory stimuli; responses were modulated by behavioural context. 2)Visual receptive fields were large (approximately 20° in diameter) and included a representation of the area centralis. 3)Maximum discharge was attained when the cat attentively fixated a visual stimulus. Discharge was reduced when the animal's attention was directed away from the point of fixation. 4)During orienting gaze shifts, discharge was reduced immediately prior to the onset of movement and increased as the gaze shift terminated on the target. These properties are similar to tonically active omnipause neurons (OPNs) that inhibit premotor neurons involved in saccade generation. We speculate that rostral TRSNs may provide excitation to OPNs, thereby suppressing orientation during attentive fixation.

386.7

NEURONAL ACTIVITY IN THE POSTERIOR PARIETAL CORTEX (AREA PG) OF ALERT MONKEY DURING OCULAR FOLLOWING RESPONSES. K. Kawano, Y. Watanabe* and S. Yamane* Bionics Section, Electrotechnical Lab., Tsukuba-shi, Ibaraki 305, JAPAN

Previous studies showed that a group of neurons in the posterior parietal cortex (area PG) of the monkey are activated by movements of the entire visual field. In this study, the experiments conducted were concerned with their relation to the ocular following responses, which is always elicited by movements of the visual field in alert monkeys. A monkey faced a screen onto which a random dot pattern was projected, and its eye movements were recorded with the magnetic search coil technique. Single unit activity was recorded in the posterior part of area PG. 166 neurons were activated by the movements of the visual scene showing directional selectivity. Most of them showed similar dependence on the visual properties of the stimulus to that of ocular following, e.g. preference to high speed (80-160°/s), latency delay due to blurring and oscillation at the high temporal frequency (40Hz). Their latencies were very short. 80% of the neurons were activated less than 50ms after the onset of the stimulus, and about half of them started their increase of firing rate more than 10ms before the eye movement. Furthermore, some of them were activated antidromically from the ipsilateral cerebral peduncle. These results suggest an important role of the neurons in the posterior part of area PG in the initiation and control of ocular following.

386.8

THE OCULOMOTOR VELOCITY TO POSITION TRANSFORMATION INVOLVES THE NUCLEUS OF CAJAL. D. Crawford*, W. Cadera* and T. Vilis. Depts. of Physiology and Ophthalmology, University of Western Ontario. London, Canada, N6A 5C1.

The mesencephalic reticular formation of Macaca fascicularis monkeys was systematically explored for evidence of an eye velocity to position signal transformation through single unit recording, microstimulation and microinjection of muscimol. Eye rotations in 3D were recorded. Constant frequency microstimulation in the vicinity of the nucleus of Cajal (INC) produced constant velocity eye rotations which held their positions when stimulation ceased. Rotations were clockwise for stimulations on the right and counterclockwise on the left with variable vertical components. As reported elsewhere (Hepp et al. 1986), unilateral injections of muscimol in the riMLF produced tonic torsional deviations of the eyes. These tonic deviations became leaky with unilateral injections between the riMLF and INC, producing nystagmus with torsional and vertical slow phases exponentially drifting toward primary position. Injections in the INC produced either no tonic deviation and thus only a vertical drift or a variable change in set point with both vertical and torsional drift. Goal directed saccades did not overshoot as expected with a loss of position feedback. The results suggest that the velocity to position tranformation involves the INC and that the position signal is not used to terminate saccades.

386.9

SMOOTH EYE MOVEMENTS ELICITED BY MICROSTIMULATION IN THE FRONTAL EYE FIELDS REGION OF ALERT MACAQUE MONKEYS. M.G. MacAvoy, C.J. Bruce, and J. Gottlieb* Sec. Neuroanatomy, Yale Univ. School of Medicine, New Haven, CT 06510.

Smooth eye movements were studied in conjunction with microstimulation of cortex near the frontal eye fields (FEF) of rhesus monkeys. Eye movements were recorded with a search coil in one eye. Both eye position and eye velocity were digitized for later analysis.

Our primary observation is that smooth eye movements, rather than saccadic, were elicited by microstimulation within a discrete region of periarcuate cortex. Smooth movements were seen using currents as low 10-50μA during both attentive fixation of stationary targets and spontaneous oculomotor behavior. These eye movements usually continued for the duration of stimulation (250-500 msec). Eye velocities up to 25°/sec were obtained, with velocity increasing as a function of current. Each site had a characteristic direction of movement. Vertical, horizontal, and oblique movements were seen. The horizontal component was almost always ipsilateral to the stimulating electrode, in contrast to the contralateral saccades usually obtained from the nearby saccadic FEF.

The region over which smooth eye movements were elicited is near the ventral (inferior) FEF region where small amplitude saccadic eye movements are elicited. It appears to be a separate zone as we seldom observed saccadic and smooth eye movements together at a single site nor interdigitating within a single electrode penetration. Instead, on most penetrations saccades were elicited superficially and then smooth movements were elicited deep in the anterior bank of the arcuate sulcus.

This phenomenon of elicited smooth eye movements suggests that the primate FEF region has specializations for directing gaze using the smooth pursuit, as well as the saccadic, type of eye movement. Properties of neurons at smooth movement sites are now being studied.

386.10

ELECTRICALLY EVOKED TURNING: ASYMMETRIC AND SYMMETRIC COLLISION BETWEEN ANTEROMEDIAL CORTEX AND STRIATUM. J.S. Yeomans and K. Buckenham*, Psychology, U. Toronto, Canada.

Stimulation of anteromedial cortex (AMC) resulted in contraversive circling mixed with ipsiversive circling in one rat. Stimulation of striatum resulted in smooth contraversive circling. In the collision test, at low currents collision was only 15%, but at high currents collision was near 40%. In both cases, summation at long C-T intervals was only 20%. Collision occurred, it seems, in the corticostriatal pathway mediating contraversive turning, but not in cortical outputs mediating ipsiversive circling. The collision was asymmetric at both low and high currents. That is, the recovery from collision was rapid (0.6 to 1.0 ms) when the C pulses were delivered to the striatum and the T pulses were delivered to AMC, but the recovery was much slower (0.6 to 4 ms) when the C pulses were delivered to AMC and the T pulses to the striatum. The rapid recovery (0.6 to 1.0 ms) was symmetric 20% collision, attributable to corticostriatal axons. The slow recovery (1 to 4 ms) was asymmetric. The asymmetric recovery is proposed to be due to activation of cortical interneurons, resulting in corticostriatal collisions that are delayed by 2 ms. The asymmetry results from the inability of antidromic action potentials from striatum to cross the synaptic barrier. The refractory period curve in AMC rose at the same long C-T intervals (2-4 ms), perhaps also due to blockade of transsynaptic action potentials.

386.11

ON COLLIDING ELECTRICALLY EVOKED AND NATURAL SACCADES. J.Schlag, M.Schlag-Rey and P.Dassonville. UCLA, BRI & Dept. Anatomy, Los Angeles,Ca.90024.

The "colliding saccade paradigm" (microstimulation applied during an ongoing saccade) differentiates brain sites processing (*a*)retinal error vs. (*b*)motor error. If (*a*) is the case, the trajectory of evoked saccades compensates for the initial gaze displacement (Exp. Brain Res. 87:68). This implies that the compensatory evoked saccades are directed toward a goal defined with respect to eye position. The time at which eye position is sampled was studied in monkeys by varying the interval between initial saccade and stimulation onsets, and testing 2 hypotheses: (*1*) the artificially created retinal error is referred to an eye position synchronous with the presumed real target onset (i.e. before the electrical stimulus), in order to compute spatial error and motor error (Robinson, 75); (*2*) the artificial retinal error is referred to the synchronous eye position (e.g. Jurgens et al., 81). Compensation for changes of eye position during afferent as well as efferent delays from the electrical stimulus is predicted by (*1*) but not (*2*). The results support (*1*) in the thalamus and (*2*) in the superior colliculus. These findings have implications for models of saccade generation. (Supported by USPHS grants EY05879, EY02305 and NSF RCD87-58034).

386.12

CHANGES IN PINNA ORIENTATION ACCOMPANY SHIFTS IN DIRECTION OF GAZE IN MONKEYS. C.J. Bruce, D. Burman, M.G. MacAvoy, and G.S. Russo. Sect. Neuroanatomy, Yale Univ. Sch. Medicine, New Haven CT 06510.

Orientation of the external ears was studied while rhesus monkeys made conjugate eye movements under controlled conditions. Eye movements were recorded with a search coil surgically implanted in one eye; pinnae movements were recorded with additional search coils temporarily mounted on the left and right ears.

Our primary observation was that both pinnae systematically oriented towards the direction of gaze. This effect was best shown by having the monkeys fixate a central LED for 1-2 sec and then saccade to a peripheral (e.g. +- 30°) LED and hold this eccentric gaze for 1-2 sec. Both ears usually moved; however, when gaze was directed to the far left the left ear moved more than the right, whereas when gaze was directed to the far right the right ear moved more than the left. Using the average of several trials, we computed a "gain" index equal to the sum of both pinnae movements (in a left-right coordinate system) divided by the horizontal gaze movement. This gain could be as high as +0.5, although it typically was nearer to +0.25. When the monkeys tracked moving visual targets via smooth pursuit eye movements the ears also moved with the eyes, showing approximately the same "gain" as with saccadic eye movements. Surprisingly, when the task was to attend to sustained peripheral sounds while maintaining fixation of a central LED there was little or no effect of the sound's location on tonic pinnae orientation. Instead, both ears transiently moved foward (left ear to the right and right ear to the left) when the sound began, regardless of its direction.

We conclude that the monkey has a basic tendency to orient its pinnae towards its direction of gaze, that is, to center of the current visual field. These pinnae movements must be considered in investigations of the brain's mechanisms for combining visual and auditory space.

386.13

THE EFFECT OF TARGET POSITION AND TARGET STEP SIZE ON SACCADE LATENCY A.D. Epstein, R.J. Tusa, N.R. Miller* Depts of Ophthalmology and Neurology, Johns Hopkins Hospital, 600 North Wolfe Street Baltimore, Maryland 21205-2182.

Horizontal saccade latency varies with target eccentricity, but it is not clear whether this relationship is based on the craniotopic or retinotopic coordinates of the target. Instead of testing only saccades made from primary gaze, which keeps target eccentricity with respect to eye and head identical, we tested saccades made from both primary and eccentric gaze positions.

We measured saccades by electrooculography in 13 normal subjects. Each was instructed to make prompt, accurate saccades to a 3 fL target light that stepped unpredictably to 1 of 7 positions between R 30° and L 30°. Subjects performed 450-900 saccades in one 60-90 minute session.

We performed multiple linear regression analysis on rightward and leftward saccades combined. Latency covaried significantly with target step size (ß=.58, p<.001) but not with initial and final target position. These results suggest that the effect of target eccentricity on saccade latency may reflect retinotopically mapped central processing. (Supported by Heed Foundation and NIH EY-07047)

386.14

PIÉRON'S LAW FOR SACCADES. H. Doma* and P.E. Hallett* (SPON: L. C. Doering), Department of Physiology, University of Toronto, Toronto, Canada. M5S 1A8.

Saccadic latencies were measured to small lit stimuli in darkness for two different tasks— foveating or anti saccades. The stimulus is the target for a foveating saccade, or is the cue for an anti saccade which peripheralizes the retinal image of the cue. Stimulus luminance, wavelength and retinal adaptation were varied. For either task Piéron's law fits pure rod or pure cone data with an exponent of -1.00, not -0.33 as others suggest. (a) For an exponent of -1.00 Piéron's law reduces to an oculomotor version of Bloch's law of temporal integration; latency is the sum of a minimal transit time from the photoreceptors to the eye muscles, plus a variable waiting or "response time" for a threshold number of photons. (b) This number for the rods is comparable to the classical perceptual limit of about 100 photons. The corresponding number for cones is roughly a factor of 5 higher than the cone threshold for perception. The implication is that rod-driven saccades are more perceptual in nature in comparison to the more reflex cone-driven saccades. (c) For foveating saccades latencies for mixed rod and cone inputs are substantially shorter than for the rods or cones alone— this is a synergistic effect. For anti saccades the same stimuli show no synergism. A neurophysiological mechanism can be proposed.

386.15

SACCADIC LOCALIZATION OF ECCENTRIC FORMS. Peiyuan He[1],*, E. Kowler[1] & R. M. Steinman[2]. [1]Rutgers Univ., New Brunswick NJ 08903 & [2]Univ. of Maryland, College Park, MD 20742.

How well can subjects direct saccades to selected locations within simple forms? Saccades were made to 1 of 4 possible locations inside an eccentric triangle 90' from fixation. The target location was indicated by a small point inside a central triangle. The eccentric triangle contained no target marker so that saccades would be programmed based only on the contour. When the eccentric triangle appeared, subjects had to make a single saccade to the designated target location as quickly as possible without sacrificing accuracy. In control trials targets were points at the same eccentricities.

Saccades to triangles were accurate (error=5') and precise (SD=11% of saccade size). The high accuracy and precision were not due to saccades learned in the point (control) trials because the same results were obtained when the triangles were tested first. Saccades directed to the "whole triangle" landed in idiosyncratic places, not the center-of-gravity. Latency was shorter and precision better than saccades to locations within the triangle.

The results do not confirm suggestions of reflexive saccades to the center-of-gravity of a form. Saccades are determined in 2 stages: (1) voluntary selection, in which a part of a form, or the whole form, is attended; and (2) automatic computation of the saccadic command based on attended visual information. (Supported by AFOSR 85-0022)

386.16

MULTI-TARGET VISUAL AND AUDITORY SACCADIC LOCALIZATION BY CATS.
D.D. Kurylo, R.L.P. Vimal*, and P.H. Hartline.
Eye Res. Inst. of Retina Foundation, Boston, MA, 02114.

Cats can direct their eyes to within a few degrees of briefly presented visual or auditory targets. We studied sensory-motor transformations underlying this behavior by investigating unimodal and bimodal interactions of auditory and visual stimuli presented at disparate locations in head fixed cats trained by an autoshaping paradigm. When two stimuli were simultaneously presented at disparate locations, most responses were single saccades either to one of the two targets, or to an intermediate position. Targets could both be visual, both auditory, or one visual and the other auditory. When the strength of one of the two stimuli was increased, the distribution of post saccade positions was shifted towards that stimulus. When the eyes were directed between the two stimuli at the time of onset, two-stimulus presentations produced responses similar to those evoked when the eyes were directed near a single target at onset time: saccadic latencies were long and the probability of eliciting a saccade was decreased. These results suggest that the neural computation of an orienting response integrates location information about multiple simultaneous stimuli, whether one or more modalities are involved. They may also reflect properties of the representation and processing of multi-sensory location information in superior colliculus.

386.17

TIMING AFFECTS MULTI-TARGET AUDITORY AND VISUAL LOCALIZATION BY CATS R.L.P.Vimal*,D.D.Kurylo and P.H.Hartline Eye Res. Inst.of Retina Found., Boston MA, 02114

Animals must be able to orient effectively to nearly simultaneous stimuli of different sensory modalities. We investigated the effects of inter-stimulus delay on saccadic orientation responses of cats trained by an autoshaping paradigm and presented with two disparate targets of auditory, visual, or mixed modality.

Saccadic latency increased, perhaps reflecting need for a complex decision, when two stimuli (same or different modality) were simultaneously presented and a single saccade to an intermediate location resulted. If one stimulus preceded the other by less than about 100 ms, a single saccade to either target or to an intermediate location resulted, indicating that decision (choice of one of the two targets) or summation occurred. At intervals < 100 msec, an initial saccade toward one target was occasionally aborted mid-stream by a saccade to a location intermediate between the two targets. For intervals > 250 msec, two sequential saccades toward the two stimulus locations often resulted, reflecting the order of stimulus presentation.

Multisensory enhancement or depression manifested by neuronal responses in optic tectum (a center for sensory motor integration for orienting behavior) can be strongly influenced by the relative timing of the stimuli. There may be complex spatiotemporal integration of tectal point images; selection of one vs integration of two competing stimuli may result from such neural interactions.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM IV

387.1

ORBITAL GEOMETRY OF THE ACCESSORY LATERAL RECTUS MUSCLE IN MACAQUE MONKEYS. R. G. Boothe, M. V. Joosse* and M. W. Quick. Yerkes Primate Research Center, Depts. of Psychology and Ophthalmology, Emory Univ., Atlanta, GA 30322.

Simians have an extraocular muscle, the accessory lateral rectus, that is not present in either great apes or humans. Its functional role in monkey eye movements is usually considered to be minor due to the fact that it is short, weak, and attaches far back on the globe (Fuchs & Luschei, J. Physiol., 1971). However, no quantitative measurements or predictions of its function are available. Recently, Miller and Robins (Vis. Res., 1987) have applied a model of the effects of muscle force on human eye movements ("SQUINT") to monkeys. Input parameters for this model are based on quantitative dissections of positions of origins and insertions, and measurements of lengths and cross-sectional areas of each extraocular muscle. Values of these parameters for the accessory lateral rectus are unknown. In order to fill in this gap and provide a basis for making quantitative predictions about the functional role of this muscle, we have made measurements of these parameters in 10 eyes of 5 monkey cadavers (4 Macaca mulatta and 1 Macaca nemestrina). Mean values (expressed in the same stereotaxic coordinates as used in Miller & Robins, 1987) are: Origin: X = -6.8, Y = -13.0, Z = -4.7. Insertion: X = -0.1, Y = -5.6, Z = 2.54. Length = 12.1 mm, width at insertion = 2.86 mm, cross-sectional area = 3.56 sq mm.

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387.2

EXTRAOCULAR MUSCLE ROTATION AXES: DETERMINATION IN THE INTACT HUMAN BY MAGNETIC RESONANCE IMAGING. E. S. Viirre*, S.J. Karlik* and T. Vilis. (spon. W.F. Brown) Depts. of Physiology and Diagnostic Imaging, University of Western Ontario. London, Canada. N6A 5C1.

As suggested by Simpson et al.(1986), contemporary data on the rotation axes of the extraocular muscles are necessary for current models of oculomotor control. The advantage of Magnetic Resonance Imaging (MRI) over dissection data is that muscle axes can be determined while the subject fixates a target straight ahead, thus giving normal innervations to each muscle.

MRI scans of both orbits were carried out on 2 adult males with no strabismus. The scanner had a 1.5 Tesla magnet which gave a resolution of approximately 0.5mm. Rotation axes, calculated using the centre of the eye and the centres of muscle cross sections, were rotated so that both medial recti were as near vertical as possible. Average direction cosines of the rotation axes are given (left eye). The reference axes are X (anterior positive), Y (left positive) and Z (superior positive) MR -.037 -.053 -.998 LR -.005 .078 .997 SR .308 -.950 -.050 IR -.339 .913 -.227 IO -.836 -.503 -.217. These results indicate that the medial and lateral recti are collinear but that the superior and inferior depart by 10-25°. This is similar to Volkmann, 1869.

387.3

LISTING'S LAW FOR THE HEAD. Douglas Tweed* and Tutis Vilis (spon. A. J. Hudson) Depts. of Physiology and Ophthalmology, University of Western Ontario. London, Canada. N6A 5C1.

If eye positions are described in terms of their rotational displacement from the normal straight ahead position, using an angular position vector which lies along the axis of the rotation and whose length is the size of the rotation, then all these vectors lie in a single plane -- a result known as Listing's law.

We have used the magnetic field/search coil technique to measure the angular position of the head in 6 normal human subjects. When instructed to look around the room, with shoulders still, 5 of the subjects showed head position vectors restricted to within 5° of a single plane -- a scatter comparable to what we have found for eye positions in humans and monkeys. The sixth subject had one plane when looking above head level and another below head level. When subjects made repetitive eye-head gaze shifts between targets at 70° eccentricity, head positions remained roughly in the plane. Small systematic deviations were attributed to motion at lower cervical joints, because they were reproduced by voluntary forward or backward bending of the neck. The existence of Listing's laws for both head and eyes shows that the law is not a consequence of muscle geometry.

387.4

HUMAN VERTICAL OPTOKINETIC NYSTAGMUS (VOKN): UP-DOWN ASYMMETRY WITH AND WITHOUT CENTRAL RETINAL STIMULATION. C. M. Murasugi* and I. P. Howard* (SPON: J. R. Mendelson). Dept. of Psychology, York University, Toronto, Ontario, Canada.

In cats and monkeys the slow-phase gain of VOKN is higher for upward than for downward stimulus motion. The presence of a consistent VOKN asymmetry in humans is controversial, probably due to use of electrooculography which produces an eyelid artifact. We used the magnetic search coil method to measure the monocular VOKN of 10 head-upright normal subjects. A 64°-high x 61°-wide random-dot display moved at velocities ranging from 10 to 70°/s. For seven subjects slow-phase gains were 40% higher in the upward direction for velocities at and above 30°/s. Three other subjects showed symmetrical VOKN. When a vertical band 6° wide spanned the center of the moving display, two patterns of results emerged. In six subjects, including one who showed a symmetrical full-field response, central occlusion caused a larger drop in downward VOKN than in upward VOKN for stimulus velocities of 50 and 70°/s. The other four subjects showed equally poor (<10°/s) VOKN in both directions. They were re-tested with a narrower occluder (3°) with little increase in evoked eye velocity. Thus, an upward dominance in VOKN is prevalent in humans at higher stimulus velocities, and the slower downward response is relatively more susceptible to the effects of central retinal occlusion. Investigations of response properties of neurons in the cat's accessory optic system have revealed that the visual cortex is a major source of upward direction-selectivity and high-velocity tuning, whereas downward direction-selective cells receive their input directly from the contralateral retina (Grasse, K.L. et al., Exp. Brain Res., 55: 69,1984). Our results suggest that human VOKN may involve similar pathways, and at higher stimulus velocities the cortically mediated upward VOKN is more vigorous and more resistant to occlusion of the central retina than the directly innervated downward VOKN.

387.5

RETINAL SLIP SERVES TO "FINE TUNE" SUPPRESSION OF OKN. M. Lustgarten*, J. Pola, H.J. Wyatt and E. Aksionoff*. Schnurmacher Institute for Vision Research, SUNY College of Optometry, NY, NY 10010.

Subjects looked at a target presented against a sinusoidally moving optokinetic inducing field (1/4 Hz). Target retinal feedback was varied from 0 (target foveally stabilized) to -1 (normal closed loop condition). With 0 feedback there was substantial suppression of OKN; however, moderate amplitude slow eye movements remained, counterphase to the field (0 feedback suppression appears to come from target-field relative motion and attention - Vis Res '84, Soc Neurosci '87). As feedback increased, amplitude and phase of this residual movement decreased, until with -1 feedback there was very little movement. The decrease was particularly rapid for small amounts of feedback presented near the stabilized condition. Linear models cannot account well for this decrease in amplitude and phase. However, a simple model with a non-linearity at its input predicts the results nicely. The non-linearity has a gain which is high near the fovea, but decreases with eccentricity from the fovea.

These findings indicate that suppression of OKN comes largely from target-field relative motion and attention, with retinal slip serving to fine-tune the suppression. The model suggests the existence of a fixation mechanism whose sensitivity is maximum at the fovea.

(Supported by NSF BNS-85-19267)

387.6

HORIZONTAL OPTOKINETIC NYSTAGMUS (OKN) IS NON-CONJUGATE IN THE TURTLE. M. Ariel. Depts. of Behavioral Neuroscience & Psychiatry, Univ. of Pittsburgh, Pgh., PA 15260.

The positions of each eye of the awake turtle, pseudmys scripta elegans, were recorded simultaneously during full-field stimuli. Horizontal OKN was strongly asymmetric and non-conjugate during monocular stimuli, with a strong response by the exposed eye to stimuli moving in the nasal direction. The occluded eye hardly moved as the exposed eye viewed nasal stimuli, but was more yoked during temporal stimuli. These dramatic differences between the eyes was also often observed during binocular viewing of identical random-dot patterns drifting horizontally, so that the slow phase velocity of the eye viewing the nasal stimulus was much greater than that of the other eye. This non-conjugate OKN is clearest to stimuli of about 10 deg/sec. Concurrent with the non-conjugate slow phases was the yoked nature of the timing of fast phase eye movements.

OKN was also analyzed simultaneously for both eyes following monocular intravitreal drug injections. Bicuculline, known to evoke spontaneous temporal-to-nasal nystagmus in mammals with yoked eyes, elicited nystagmus mainly in the injected eye of the turtle. Likewise, monocular injections of APB, known to eliminate OKN in rabbits, exclusively blocks OKN of the injected turtle eye, without affecting the eye movements of the uninjected eye. Therefore, monocular OKN and effects of monocular drugs indicate that oculomotor pathways in the turtle can remain functionally independent for each eye.

Supported by USPHS EY05978 and MH30915.

387.7

COMPARISON OF VESTIBULO-OCULAR REFLEX (VOR) MODIFICATION METHODS IN CATS. W. Freedman, J.R. Carroll*, and J.G. McElligott. Drexel Univ. Dept of Electrical and Computer Engineering, Phila., PA 19104, Pennsylvania College of Optometry, Phila., PA, Temple University School of Medicine, Dept. of Pharmacology, Phila., PA 19140, and Moss Rehabilitation Hospital, Phila., PA.

The vestibulo-ocular reflex (VOR) has been measured and optically modified in several animal species. VOR gain can be increased optokinetically by placing a head fixed animal inside an optokinetic drum and sinusoidly oscillating it in a direction opposite to that of the drum rotation. VOR increase can also be accomplished by having an animal wear a set of magnifying lenses. We describe here a comparison of three methods for producing VOR increases in cats using an (i) optokinetic drum, (ii) a pair of 2.2X telescopic lenses, or (iii) a Fresnel lens goggles that we have recently developed in our lab. After initial calibrations to test the VOR in the dark and the light, 10 VOR modification periods of 15 minutes were each followed by a 1 minute period to test for VOR gain modification in the dark (see McElligott and Freedman, *Exp. Brain Res.* (1988) 69:509-521). Throughout the modification and the test periods, each animal was kept in a highly alerted state. The results of the comparison in 4 cats show that the Fresnel lens system produces a greater and more stable VOR gain increase than the other two systems. After 2.5 hours of modification, the VOR gain increases (n=4) were optokinetic drum- 1.15; 2.2X telescopes- 1.25; Fresnel lens- 1.40. These results show that some animals that had poor or minimal VOR modification, now exhibit more robust VOR gain increases using the Fresnel lens system.

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387.8

BEHAVIOR OF HUMAN VESTIBULO-OCULAR REFLEX (VOR) IN RESPONSE TO HIGH-ACCELERATION STIMULI. E.F. Maas,* W.P. Huebner,* S.H. Seidman* and R.J. Leigh, Ocular Motility Lab Cleveland V.A. Med. Ctr., University Hospitals and Dept. Biomed. Eng. Case Western Reserve University, Cleveland, OH, 44106.

We measured horizontal head position and gaze in 3 normal male subjects (age range 23-30 yrs) using a magnetic search coil system. Each subject clenched his teeth upon a plastic mouthguard attached to a lucite yoke that encircled his head. At the back of the yoke was a spur that was struck in a non-predictable sequence to generate small magnitude ($\lesssim$3.5 deg) but high acceleration (2000-7000 deg/sec/sec) head rotations. Data were filtered (0-150 Hz) and digitized at 2 KHz. Head velocity and eye-in-orbit velocity were then obtained for 10 trials for each subject.

The latency of the VOR (onset of head rotation to onset of eye-in-orbit rotation was $\leq$ 12 msec, which is shorter than values reported with lower acceleration stimuli in monkey. Peak gaze velocity (group mean) was 30 deg/sec, which was 45% of peak head velocity; oscillopsia occurred during each head perturbation. The mean, predominant frequency of head rotations was 11 Hz. These results are relevant to the performance of the VOR during running, when head rotations of similar amplitude and acceleration may occur.

(Supported by NIH grant EY06717, NASA contract 9-17439 and the Veterans Administration)

387.9

Temporal Characteristics of Vestibulo-ocular Reflex Cancellation in Squirrel Monkeys. K. E. Cullen, T. Belton*, L.B. Minor, and R. A. McCrea. Comm. on Neurobiol., Univ. Chicago, Chicago, Ill. 60637.

The temporal characteristics of the central systems used to cancel the vestibulo-ocular reflex (VOR) were studied in squirrel monkeys who were trained to fixate a target that moved with the head. Monkeys were passively rotated on a vestibular turntable in the horizontal plane. Two types of experiments were done: 1) The table was turned at a constant velocity of 20°/sec during VOR cancellation, and was then rapidly accelerated to a new velocity of either 50°/sec or -10°/sec. This unpredicted change in head velocity produced a transient eye velocity in the opposite direction. The transient had a duration of 140-210 msec, after which the eye velocity returned to zero. The gain of the VOR during the transient response was less that one, in respect to the step change in head velocity, after a delay of 10-15 msec. 2) A second series of experiments were designed to determine the frequency response characteristics of the cancellation system. The monkeys were rotated sinusoidally at a low frequency (0.1-0.9 Hz), and once eye velocity was cancelled, the frequency of rotation was increased to 1.0-6.0 Hz. VOR gain was zero for stimulus frequencies below 1.2 Hz during cancellation, but was near 1.0 for stimulus frequencies above 4.0 Hz. Thus, the failure of the cancellation system to completely cancel the VOR during unexpected changes in head velocity probably reflects the fact that the input to the cancellation system is only a low pass filtered copy of the signals that drive the VOR.

387.10

HUMAN SUBJECTS SUPPRESS THE VESTIBULO-OCULAR REFLEX DURING VISUAL PURSUIT. V. Matsuo and B.W. Peterson. Sensory Motor Performance Program, Rehabilitation Institute of Chicago, and Department of Physiology, Northwestern University School of Medicine, Chicago, IL 60611.

We have shown previously that when rotating subjects (Ss) pursue a moving target their maximum smooth eye velocity is less than the sum of their VOR and smooth pursuit responses when measured independently. We now report that this is because Ss suppress the VOR when pursuing moving targets. This occurs when Ss pursue a target while they are initially stationary and then are suddenly rotated (condition 1) , as well as when Ss pursue the target during rotation and are suddenly stopped (condition 2) .

Five normal Ss sat in a servo-controlled rotating chair with the head held in a chair-mounted restraint. Eye movements were recorded using d.c. EOG. Head velocity was monitored using a head-fixed angular rate sensor. The pursuit target was a 0.25° projected laser spot. Vestibular and pursuit stimuli consisted of computer-generated 20 and 40 °/sec constant velocity ramp waveforms.

Our criterion for VOR involvement was the latency from change in head velocity, due to the sudden rotation or stop, to change in eye velocity. For 40°/sec median VOR latency was 38 msec (range 12-59). Eye movement latencies greater than 59 msec were thus regarded as putative evidence that the VOR had been suppressed. Group median latency to eye velocity change for condition 1 was 104 msec (medians for the 5 Ss ranged from 69-208). The median latency resulting from condition 2 was 64 msec (range from 56-76). The Mann-Whitney U-test showed greater eye movement latencies during conditions 1 and 2 than during VOR in darkness ($p<0.01$ for all Ss). In three Ss the latencies for condition 1 were significantly greater than those for condition 2 ($p<0.005$), suggesting that the VOR was at least as effectively suppressed when S was initially stationary as when he was moving. Results were similar at 20°/sec except that there were no differences between conditions 1 and 2.

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387.11

THE ANATOMICAL SUBSTRATE OF BLINK MOVEMENTS: STRUCTURE AND INNERVATION OF PRIMATE LEVATOR PALPEBRAE SUPERIORIS AND ORBICULARIS OCULI MUSCLES. J.D. Porter, P.J. May, and L.A. Burns*. Dept. of Anatomy, Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

The levator palpebrae superioris (LPS) and orbicularis oculi (ObOc) are antagonistic muscles functioning in blinks. Motoneurons innervating these muscles were identified in Cynomolgous monkeys by retrograde transport of HRP. LPS motoneurons were located bilaterally within the caudal central division of the oculomotor nucleus. The lack of any apparent laterality in motoneuron distribution and the high percentage of neurons labeled from injection of one LPS suggest that individual motoneurons may innervate both LPS muscles. Motoneurons innervating the ObOc muscle were distributed within the dorsolateral subdivision of the ipsilateral facial motor nucleus, with a few neurons in the corresponding locus of the contralateral nucleus.

Unlike the other extraocular muscles, the LPS lacks a layered distribution of fiber types. The LPS contains the same 3 ultrastructural types of singly-innervated muscle fibers (SIF) found in the global layer of other extraocular muscles. Fiber types corresponding to extraocular muscle global red SIF (type 3) and global intermediate SIF predominate (type 4), with only a few global pale SIF fibers (type 5) noted. The histochemical/morphological profiles of these fiber types are such that they do not respect "traditional" fiber classification schemes, but are consistent with a role for LPS in tonic elevation of the lid. The multiply-innervated fiber types, which characterize eye muscles, were absent from the LPS, suggesting that this fiber's functional role relates to rotational movements of the globe and not to maintenance of lid position against gravity. The ObOc exhibited two morphological fiber types that, by contrast, resembled "traditional" skeletal muscle fast-twitch fibers. On the basis of mitochondrial content, ObOc fiber types would be fatigue-prone; an assumption consistent with their rapid onset/offset action in blinks. Morphological differences between LPS and ObOc reflect not only their distinct functional roles in blinking, but also their diverse embryological origins. NEI EY05464 (JDP) and EY07166 (PJM).

387.12

CEREBELLAR PARTICIPATION IN ADAPTIVE MODIFICATION OF THE BLINK REFLEX. C. Evinger and K.A. Manning. Dept. Neurobiology. & Behavior, SUNY Stony Brook, Stony Brook, NY 11794.

Blink reflex size as measured by EMG activity exhibits rapid, adaptive modification following restraint of the eyelid. Using lesions and electrophysiological techniques on decerebrate rats, we investigated the role of the cerebellum in adaptive changes of the blink reflex.

After hemicerebellectomy, the eyelid ipsilateral to the lesion did not exhibit adaptive modification with eyelid restraint. The contralateral eyelid, however, continued to adapt. Thus, the cerebellum appears to be necessary for adaptive modification of the blink reflex. Single unit recording in Crus I revealed that corneal stimulation and the resultant blink normally evoke a complex spike and a transient increase in the simple spike activity of Purkinje cells. During adaptation induced by lid restraint, complex spike activity decreased and simple spike firing frequency increased for most Purkinje cells. Although these results imply that the cerebellum is the site of adaptation, mossy fibers whose discharge correlated with corneal stimulation also showed an increased activity during adaptation. Since the mossy fiber input to the cerebellum changes with adaptation, the cerebellum may be providing a signal to initiate blink adaptation, rather than serving as the site of adaptation.

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387.13

AN INEXPENSIVE ULTRASONIC CONTACT LENS TO MEASURE ACCOMMODATION. K.Kozonasky and R. Remmel, Biomedical Engineering Dept., Boston University, Boston, MA 02215.

Optical methods of measuring accommodation, some costing >$10,000, involve observing how a light is focused on the retina. An ultrasonic probe placed against the eye can measure lens thickness. These methods obstruct or restrict vision, possibly biasing the results. Our method measures lens thickness with two ultrasound crystals mounted on a contact lens, leaving vision unobstructed.

The two lead metaniobate crystals are 0.5 mm thick and 1 mm dia. They will be placed on either side of the pupil. The transmitting crystal is excited by a 50 V step, which causes a 6 MHz oscillation with a decay time constant of 2 us. The receiving crystal detects the direct wave traveling across the cornea, and then the echoes from the front and back lens surfaces. In the finished circuit, the time between echoes will be converted to a voltage proportional to lens thickness, which measurement will be repeated 2800 times/s.

Distances as short as 2.5 mm have been measured with 8% accuracy in oil. Testing is underway using cat eyes.

A silicone-rubber contact lens with a hole over the pupil is being tested. The two crystals can be glued on with silicone rubber glue. A local anesthetic should be applied to the eye. A coil of wire can also be glued to the lens so that eye movements can be measured.

Accommodation should thus be measureable for $300.

DRUGS OF ABUSE IV

388.1

DOSE RESPONSE EFFECTS OF COCAINE ON EVOKED ACTIVITY RECORDED FROM DORSAL RAPHE AND PARABRACHIAL AREA. W. McVaugh*, A. Shen* and N. Dafny (SPON: A. Schonbrunn). Dept. Neurobiol. & Anat., The Univ. of Texas Medical School at Houston, 77225.

Cocaine has been reported to have variable effects on CNS components. This study investigated the effects of cocaine on Sensory Evoked Responses (SERs) recorded from Dorsal Raphe (DR) and Locus Ceruleus (LC) in the freely moving rat. In addition, the effects of Naloxone (an opiate antagonist) and Desipramine (a NE uptake blocker) were examined. Sixteen male Sprague-Dawley rats were permanently implanted with 120 μm stainless steel semimicroelectrodes. Four sets of 32 averaged visually evoked responses were recorded before (control), and after 1, 5 and 10 mg/kg cocaine (i.p.) and desipramine (20 mg/kg) or naloxone (1 mg/kg), respectively. The P_2 and P_3 wave components were analyzed. In LC, the P_2 amplitude increased with each increasing dose. P_3 exhibited facilitation at 1 mg/kg, but was inhibited at higher doses. P_2 from DR demonstrated a dose-dependent facilitation, while P_3 was inihibited. Naloxone had no effect in either structure, while treatment with desipramine augmented the response from LC but not DR. In conclusion, it was seen that (1) cocaine affects various CNS sites differently in dose-dependent patterns; (2) cocaine can differentially affect the individual components of the SER; and (3) cocaine's effects do not seem to be mediated via opiate receptors.

388.2

EFFECTS OF SEROTONERGIC LESIONS OF THE NUCLEUS ACCUMBENS ON THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE. P. Schauwecher*, C. Bimle*, R. Kaltenbach* and S.I. Dworkin (SPON: R.D. Brown). Depts. of Psychiat. and Pharmac. & Ther., LSU School of Medicine, Shreveport, LA 71130.

Neurotoxin lesions of discrete brain regions have been helpful in determining the involvement of specific neuronal systems in drug abuse. The neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) which destroys neurons containing serotonin, increased amphetamine self-administration when injected intraventricularly. However, a localized lesion of the nucleus accumbens did not alter drug intake (Lyness et al., 12, 937-941). It is difficult to assess the effects of a lesion on the reinforcing efficacy of a drug by the use of a single behavioral procedure thus, the effects of a 5,7-DHT lesion of the nucleus accumbens on cocaine drug discrimination was determined.

Adult male Fischer-344 rats were trained to discriminate cocaine (10 mg/kg, IP) from saline using a standard drug discrimination paradigm. Subjects were then tested using a cumulative dosing procedure. After the cocaine discrimination was acquired, generalization gradients were determined. Rats were then lesioned with the neurotoxin and generalization gradients were redetermined. The neurotoxin lesion resulted in a modest attenuation of the discriminative stimulus properties of cocaine. Supported By USPHS Grant DA-03631.

388.3

TOXIC CONSEQUENCES OF COCAINE ARE AUGMENTED BY NONCONTINGENT DRUG ADMINISTRATION. S.M. Dworkin*, C. Volkmer* and S.I. Dworkin. Dept. of Psychiatry, LSU School of Medicine, Shreveport, LA 71130.

The pathological consequences of environmental events can depend on the functional relationships between behavior and the delivery of the event. Studies using both monkeys and rats in yoked procedures have shown that yoked animals are more likely to develop ulcers and other other signs of physiological and neurobiological pathology compared to animals given the opportunity to postpone the stimulus. Additionally, the contingent delivery of intravenous morphine produced greater changes in neurotransmitter turnover compared to noncontingent infusions.

Twelve littermate triads of male Fisher F-344 were prepared with chronic indwelling intravenous catheters and placed in individual operant conditioning chambers. Each set of 3 operant conditioning chambers were housed in single sound-attenuating enclosure. Responses by the rat placed in the center of the enclosure resulted in the delivery of cocaine infusions (1.0 or .67 mg/inf) to both the center rat and his littermate on the right, while saline was infused to the animal on the left. Cocaine resulted in the death of only the rats receiving noncontingent injections within a relatively short time period.

Research Supported by NIDA Contract# 271-87-8118

388.4

HEMODYNAMIC RESPONSE CHARACTERISTICS TO COCAINE (C) IN RATS. M.M. Knuepfer, D.M. Wehner* and T.L. Sellers*. Dept. Pharmacol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Our laboratory has reported that C produces small, dose-dependent increases in arterial pressure (AP) and mesenteric vascular resistance (MVR) and little change in hindquarter resistance (HQR) in awake rats. We sought to describe the mechanisms by which the hemodynamic responses were mediated using receptor antagonists. Rats were instrumented with an arterial cannula for AP and heart rate (HR) determination and with miniaturized pulsed Doppler flow probes for estimating changes in HQR and MVR. After recovery, C was administered alone or 10 minutes after pretreatment with selective antagonists. The table contains the results expressed as mean ± SEM (N's = 4-12).

DRUG (mg/kg)	AP	HR	HQR	MVR
Cocaine (5)	14±3	-35±13	-3±8	44±20
Pentolinium (7.5)	12±3	11±10	8±6	17±6
Methylatropine (1)	4±2	-27±5	-20±13	13±4
Prazosin (0.1)	-2±2	-16±8	-13±1	9±6
Propranolol (1)	21±3	-69±7	0±8	53±21

These data suggest that the modest AP and MVR responses are dependent upon α_1-adrenergic receptors, that the HR response is due primarily to removal of central sympathetic tone (α_1-adrenergic mediated) and that β-receptor blockade, often used clinically to treat C toxicity, actually exacerbates AP and HR responses to C. (Supported by HL37224, HL38299 and AHA, MO Affiliate.)

388.5

COCAINE KINETICS AND DISTRIBUTION IN VARIOUS BRAIN REGIONS OF RAT. R.P. Sharma*, J.I. Javaid, B. Duslak* and J.M. Davis*. Illinois State Psychiatric Institute and University of Illinois at Chicato, Il 60612

It has been proposed that the effects of psychoactive drugs on specific aspects of mental function may be related to the capacity of the drug to selectively concentrate in specific regions of the brain. In rat brain cocaine effects on striatal and nucleus accumbens dopaminergic systems show quantitative differences. In the present studies we report single-dose kinetics in serum and various brain areas of the rat after 10 mg/kg I.P. injection. At different time points after drug administration (5, 10, 20, 30, 60 and 120 minutes), the animals (N = 6 at each time point) were decapitated and cocaine was quantitated in serum and in various brain regions. There were large inter-individual variability in different rats in various kinetic parameters. The average serum $t_{1/2}$ was 50 minutes. There were no significant differences in the total concentration (ng/g tissue) of cocaine in the eight brain regions studied. Similarly the maximum concentration (C_{max}) in various brain regions was also not statistically different. However, the time to achieve maximum concentration (T_{max}) divided the brain regions into two groups; those with T_{max} under 10 minutes and those with T_{max} over 30 minutes. These differences in time to reach maximum drug concentration may account for differential biochemical effects in various brain areas.

388.6

A RELATIONSHIP BETWEEN WITHDRAWAL AND TOLERANCE TO THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE. M.W. Emmett-Oglesby, D. M. Wood, D. A. Mathis*, and H. Lal. Department of Pharmacology, TCOM, Fort Worth, TX 76107.

This experiment tested whether tolerance to cocaine was related to cocaine dependence/withdrawal. Animals trained to detect the discriminative stimulus properties of pentylenetetrazol (PTZ) can be used to assay for the occurrence of withdrawal from a variety of drugs of dependence, including cocaine. In addition to producing a PTZ-like stimulus, termination of high-dose cocaine administration is also associated with tolerance to the discriminative stimulus properties of cocaine, suggesting that the occurrence of a PTZ-like stimulus during withdrawal may be a mechanism that produces the apparent tolerance to cocaine. To test this hypothesis, we trained one group of rats to detect the anxiogenic drug pentylenetetrazol (20 mg/kg), and a second group of rats to detect cocaine (10 mg/kg). Given acutely, 1) PTZ shifted the dose-effect curve for the detection of cocaine approximately 2-fold to the right; 2) diazepam did not antagonize or potentiate the cocaine cue; and 3) diazepam (5 mg/kg) antagonized the PTZ cue. Following these tests, cocaine-trained subjects were given cocaine, 20 mg/kg, every 8-hr for 7 days. Subsequently, the cocaine dose-effect curve was redetermined both alone and in combination with diazepam, 5 mg/kg. Subjects receiving cocaine alone were tolerant, showing approximately a 2-fold shift of the dose-effect curve to the right. In contrast, subjects tested with combinations of cocaine and diazepam showed no shift of the dose-effect curve. These data show that diazepam blocks the expression of tolerance to the discriminative stimulus properties of cocaine. In combination with the other results, the most likely explanation for this finding is that termination of high-dose cocaine generates a PTZ-like stimulus that antagonizes the discriminative stimulus produced by cocaine.

388.7

EFFECTS OF PRENATAL EXPOSURE TO COCAINE OR RELATED DRUGS ON RAT OFFSPRING DEVELOPMENT AND DOPAMINERGIC NEUROTRANSMISSION. M. G. Henderson* and B. A. McMillen (Spon. by J. P. DaVanzo), Dept. Pharmacology, Sch. of Medicine, East Carolina University, Greenville, NC 27858.

Gestating rats received 15 mg/kg b.i.d. cocaine, 1.5 mg/kg amfonelic acid (AFA), 10 mg/kg amitryptiline or saline daily throughout pregnancy: cocaine treated dams gained less weight than controls. Male pups from 3 dams were fostered by 2 surrogate mothers, 10 per litter. Cocaine treatment accounted for 2 of 4 still births and all 4 non-fatal birth defects. There were no differences across groups in average birth weight, or weight at 15 or 30 days of age except for AFA exposed pups. All 4 groups had eye opening at the same age, but the cocaine exposed pups took 8.2 days to gain righting reflex vs 6.5 days for saline groups ($p<0.01$). At 30 days of age spontaneous motor activity before and after lights out was similar across groups. These results confirm a report by Richardson and Verhage (Fed. Proc. 2:A1804, 1988). There was no difference in akinetic response to 0.3 mg/kg s.c. haloperidol and no differences in striatal D2 receptor Bmax or Kd values. Data on brain area monoamine metabolism and later developmental ages will be described. These preliminary results indicate that the non-teratogenic effects of cocaine are small and that hyperactivity seen after prenatal amphetamine exposure does not occur. Whether significant effects may become apparent at later ages is yet to be determined.

388.8

SUBCORTICAL BINDING OF COCAINE IN LIVING BABOON BRAIN USING PET. J. S. Fowler, A. P. Wolf*, S. L. Dewey, R. R. MacGregor*, D. J. Schlyer*, D. Christman*, B. Bendriem*, N. Volkow. Brookhaven National Laboratory, Upton, NY 11973.

The mood elevating and euphoric properties of cocaine coupled with its widespread abuse and highly addictive properties has stimulated the study of the neurochemical basis of its behavioral effects. Recent evidence links the behavioral effects of cocaine to its interaction with the dopamine transporter (Ritz et al,Science 237: 1219, 1987). We recently synthesized [N-^{11}C-methyl]cocaine and measured its regional and temporal distribution in living (anesthetized, ketamine, isofluorane) baboon brain with high resolution PET. Uptake was highest in subcortical structures bilaterally, peaking at 4 minutes and clearing to 30 % of the highest value by 30 min. Ratios of striatum to frontal cortex and cerebellum at peak uptake were 2.0 and 2.4 respectively. Plasma clearance of carbon-11 was rapid and at 30 minutes, more than 50 % of the radioactivity was labeled carbon dioxide. The high initial uptake of [^{11}C]cocaine into the brain parallels the rapid mood elevation produced by the drug. The striking regional binding to the striatum suggests that the neurochemical mediator of behavioral activation may reside in subcortical structures. Research supported by USDOE, OHER and NIH NS-15638.

388.9

EVIDENCE FOR A BIPHASIC RESPONSE TO COCAINE IN RATS. L.E. Baker and R.J. Barrett. Dept. of Psychology, Vanderbilt University and Veterans Administration, Nashville, TN 37240.

Using a two choice drug discrimination paradigm, rats (n=72) were trained to discriminate cocaine (1.0 mg/kg) from haloperidol (.025 mg/kg). The training doses were chosen such that when Ss were tested on saline, they responded about 50% on each lever. A dose-response function was established by testing independent groups with three doses of cocaine (1.0, .50, .25 mg/kg) and three doses of haloperidol (.025, .0125, .006 mg/kg) during 5 min extinction sessions.

Ss were then injected with a single dose of cocaine (20 mg/kg) and independent groups were tested either 4, 6, 8, 12, 16, 18 or 24 hours later. Percent responding on the cocaine lever gradually decreased over time, such that by 8 hrs, responding was about equal on both bars and by 16 hrs, responding on the cocaine lever was 38%. Over longer intervals, there was a gradual return to pre-drug baseline.

With a single injection of 40 mg/kg cocaine, a similar biphasic effect was observed. At 24 hrs post cocaine, the Ss made 17% of their responses on the cocaine lever (i.e. 83% on haloperidol lever) before returning to predrug baseline.

These data provide evidence for a post-cocaine withdrawal, during which subjects respond in a manner opposite that produced by the primary drug effect. We believe the two drug-drug discrimination paradigm is an appropriate animal model for the study of physiological substrates which mediate the biphasic effect of cocaine in humans.

388.10

EFFECTS OF CHRONIC COCAINE TREATMENT ON THE UPTAKE AND RELEASE OF STRIATAL DOPAMINE. S.-J. Yi* and K.M. Johnson, Dept. of Pharmacology and Toxicology, Univ. of Texas Medical Branch, Galveston, TX 77550.

This laboratory has recently shown that repetitive administration of cocaine enhances its acute behavioral effects, and this behavioral sensitization involves the striatal dopaminergic system. (Soc.Neurosci.Abst.13:1718)

Female Sprague-Dawley rats were injected with either saline or 15 mg/kg cocaine (i.p.) twice a day for 7 days. After 7 days of withdrawal, rats were injected with saline or cocaine and were sacrificed 30 min later. Brains were removed and striatum was dissected to assay ^{3}H-DA uptake, ^{3}H-GBR 12935 binding and synaptosomal ^{3}H-DA release. Cocaine-sensitized rats showed an increased V_{max} of ^{3}H-DA uptake without any change in K_m, and no detectable difference in total specific 3H-GBR binding to striatal membranes or in the IC_{50} of cocaine to displace ^{3}H-GBR binding. Further, neither acute nor chronic cocaine administration caused any effects on compartmentalization of ^{3}H-DA in striatal synaptosomes. However, when 1 μM amphetamine (Amp) was added to the superfusion buffer, Amp-induced release of DA from the vesicular pool was potentiated by acute cocaine treatment. Treatment with chronic cocaine prevented this potentiation, and retarded release from the cytoplasmic, readily releasable pool, suggesting that the processes responsible for mediation of Amp's effect on DA compartmentalization show compensation in response to chronic cocaine.

388.11

PHYSIOLOGY OF ABUSE POTENTIAL SUBSTANCES IN CENTRAL NEURONAL CIRCUITS: COCAINE EFFECTS ON SOMATOSENSORY AND CEREBELLAR NEURONAL RESPONSES TO IONTOPHORETICALLY APPLIED GLUTAMATE. C.A. Jimenez-Rivera and B.D. Waterhouse. Dept. of Physiol. and Biophys., Hahnemann Univ., Phila., PA, 19102-1192

Cocaine's psychostimulant and reinforcing properties, are well known. However the basic physiological mechanism(s) through which cocaine produces these effects have not been established. Biochemical studies indicate that cocaine can increase central synaptic levels of monoamines through blockade of reuptake mechanisms. Previous work from our laboratory has suggested a modulatory role for norepinephrine (NE) in the cerebral cortex and cerebellum of mammalian brain. The goal of the present investigation was to determine to what extent cocaine might exert similar modulatory actions in neuronal circuits with well characterized noradrenergic projections. Somatosensory and cerebellar unit activity was recorded from halothane or urethane anesthetized rats. Excitatory responses of individual neurons to microiontophoretic pulses (10 sec.) of glutamate (1-65nA) were examined, before, during and after cocaine iontophoresis. Application of cocaine to somatosensory cortical cells produced a dose dependent range of effects; from marked potentiation of glutamate-induced excitation to suppression of both spontaneous and evoked activity. At lower ejection currents (less than 30nA) cocaine's major effect was to enhance glutamate responses (58%, n=12) by either an absolute potentiation of evoked discharge (25%) or by a relatively greater suppression of spontaneous versus transmitter-induced activity (33%). At higher doses (greater than 30nA), cocaine exerted a net depressant effect on both glutamate evoked and spontaneous firing (71%, n=7). A similar range of dose dependent effects was observed in cerebellum, however no absolute potentiation of evoked discharge was observed. Cocaine's facilitating effects on glutamate responses were fourfold greater and more consistent than those mediated by procaine. Overall, these data indicate that cocaine can mimic previously observed modulatory actions of NE and as such may facilitate synaptic excitatory transmission within the cerebral cortex and cerebellum. Such actions in these and other noradrenergic target circuits of the CNS could provide a physiological basis for cocaine's psychostimulant properties. (Supported by AFSOR-87-0138 & NS18081 to B.D.W.).

388.12

COCAINE ALTERS SENSORY RESPONSIVENESS AND FUNCTIONAL CONNECTIONS WITHIN NETWORKS OF SIMULTANEOUSLY RECORDED NEURONS IN THE SI CORTEX AND VPL THALAMUS OF BEHAVING RATS. C.-H. Shin, C. Jimenez-Rivera, B.K. Jin*, B.D. Waterhouse, J.K. Chapin Hahnemann Univ., Physiol., Phila. PA. 19102

To define the neural circuit basis for cocaine's effect on cognitive and motor functions of the brain, ensembles of single neurons were recorded simultaneously in the somatosensory cortex (SI) and thalamus (VPL) of freely moving rats. Up to 12 single neurons were recorded simultaneously through 25μm microwires implanted in the forepaw regions of the SI cortex and/or VPL thalamus. Movement dependent changes in sensory transmission were tested by generating post-stimulus time histograms of neuronal responses to forepaw stimulation through implanted electrodes, during treadmill locomotion (10 s ON/ 10 s OFF). Single unit responses were recorded for 10 min. before, and 40 min. after cocaine administration (0.25, 1.0 or 10 mg/kg; i.p.). Procaine (1.0 and 10.0 mg/kg) as well as saline were tested in control experiments. Cocaine at 1.0 mg/kg facilitated neuron responses to forepaw stimulation in VPL thalamus and SI cortex during both rest and movement, and thus, counteracted movement-induced suppression of sensory responsiveness. By contrast, higher doses of cocaine(10.0 mg/kg) suppressed sensory responses at rest and also enhanced the suppression of sensory responses caused by movement. Saline as well as the lowest dose of cocaine (0.25 mg/kg) did not alter the magnitude of sensory responses during rest or running. Procaine primarily exhibited depressant effects on sensory transmission. The simultaneous recording of many-neuron ensembles also allowed study of latency relationships and functional synaptic interactions, through use of spike-triggered histograms. Cocaine was found to alter routing of sensory information within a neuronal ensemble and also abolished the cyclic co-firing of cortical neurons that was normally observed during rest. **In conclusion,** our study initiates a major new avenue of investigation for drugs of abuse in awake animals and, moreover, these results are in good agreement with the dose-related modulatory effects of cocaine on synaptic transmission as observed in anesthetized rats. Supported by AA00089, AA06965, and NSF BNS-8419579 to JKC, AFOSR-87-0138 and NS18081 to BDW.

388.13

COMPARING EFFECTS OF KETAMINE, ETHANOL, AND BARBITURATES ON SINGLE SOMATOSENSORY CORTICAL NEURONS IN BEHAVING RATS. I.M. Patel and J.K. Chapin, Hahnemann University, Philadelphia, PA 19102

This study defined the differential effects of ketamine, ethanol, and barbiturates on ensembles of somatosensory (SI) cortical neurons simultaneously recorded in behaving rats. Arrays of 25μ microwires (8) were chronically implanted in the SI cortical forepaw region. Discriminated single units from each wire could be recorded for time periods (several days or weeks) sufficient to allow multiple drug experiments to be carried out on the same group of neurons. Neurons were categorized according to their sensory and behavioral properties, and also their dose responses to central administration of different drugs. Treadmill running (5 sec ON- OFF) was used as a standard means of measuring neuronal discharge during rest and movement behavior. Subanesthetic doses of ketamine (5-50 mg/kg,I.M.) caused clear firing rate increases in 35, and decreases in 24 of the total 66 neurons which were used in multiple drug experiments. While these doses produced locomotor hyperactivity, confusion and disorientation, this hyperactivity could not explain the firing rate increases of the 11 neurons which normally fired more slowly during movement. Also, since ketamine markedly reduced the sensory responsiveness of cortical neurons, increases in activation of sensory receptors during movement could not cause the increases in cortical activity. Ethanol (1.6-2.4 g/kg, 20% in normal saline, I.P.) produced moderate to severe intoxication, and supression of spontaneous discharge and evoked sensory responses of all the recorded SI cortical neurons, many of which were excited by similarly analgesic doses of ketamine. While neuronal discharge rates recovered from ketamine in 1-2 hours, full recovery from ethanol required up to 8-12 hours of a single dose. Pentobarbital at subanesthetic doses (15-25 mg/kg, I.P.) also produced universal decreases in neuronal firing rates, which persisted for more than 12 hours. These doses also blocked ketamine-induced behavioral and neuronal excitation, whereas ethanol did not. The ketamine excitatory effects were also more prone to development of tolerance (clear after 3 daily exposures). No such quick tolerance was observed in the depressive effects of either of the three drugs. *Supported by UPHS grants AA00089 and AA06965, and NSF grant BNS18041 to JKC.*

388.14

POSSIBLE GENETIC PREDISPOSITION TO COCAINE TOXICITY. R.B. MILLER,* S. HOWARD AND C.L. BLANK, Dept. of Chemistry, Univ. of Oklahoma, Norman, OK 73019.

The normal human metabolism of cocaine involves ester cleavage by serum and other cholinesterases (Stewart et al., Life Sci., **20**, 1557 (1977)). Thus, the existence of genetically atypical individuals should cause concern with respect to their ability to withstand rapidly repeated doses of cocaine, as is commonly encountered in the abuse of this substance. This is of particular concern for individuals who are reported to be homozygons for the so-called "silent" gene. Such persons exhibit little or no butyrylcholinesterase activity (Doenicke et al., Proc. Eur. Congr. Anaesthesiol., **2**, 187 (1962); Liddell et al., Nature, **193**, 561 (1962).

We, thus, decided to examine the tolerance of mice to repeated doses of cocaine following pretreatment with a relatively selective inhibitor of serum cholinesterase, diisopropylfluoryl phosphate (DFP; Foldes et al., Clin. Pharmacol. Ther., **7**, 620 (1966)). In one such test, controls (n=8) were treated 24 hr. prior to cocaine with isotonic saline while experimentals (n=7) received 6.3 mg/kg DFP, i.p. Both groups were subsequently repeatedly injected with cocaine (15 mg/kg, i.p., every 5 min). The mean (±SEM) number of injections required to cause expiratin was: controls, 8.5±1.8; experimentals, 3.9±1.1 ($P<0.001$).

388.15

EVIDENCE OF A GABA/BENZODIAZEPINE MECHANISM MEDIATING THE PENTYLENETETRAZOL-LIKE STIMULUS PRODUCED DURING COCAINE WITHDRAWAL. D.M. Wood, P.R. Laraby and H.Lal, **Department of Pharmacology, Texas College of Osteopathic Medicine, Ft. Worth, TX, 76107**

Chronic cocaine users often experience bouts of anxiety and panic attacks following termination of the drug. This effect has been quantitatively assessed in experimental animals with drug discrimination methodology using an anxiety-producing drug, pentylenetetrazol (PTZ), as a discriminative stimulus (Wood and Lal *Life Sci.* **41:1431**, 1987). In the present experiment, rats were trained to discriminate the stimulus properties of PTZ, 20 mg/kg, using a food-rewarded 2-lever choice task. Responding under an FR10 schedule was reinforced on one lever following PTZ injection and on the other lever following saline injection. Following training, substitution tests were performed, and rats selected the PTZ-appropriate lever after PTZ in a dose-dependent manner, but not after cocaine. Subsequently, testing and training were halted, and cocaine, 20 mg/kg/8-hr, was administered for 7 days. Chronic cocaine injections were then terminated and spontaneous withdrawal was assessed by determining if saline would substitute for PTZ. The rationale for this test was that if withdrawal from cocaine produces a PTZ-like stimulus, then subjects injected with saline would select the PTZ-lever rather than the saline-lever. Cocaine withdrawal progressively substituted for the PTZ stimulus reaching a peak (83% PTZ-lever selection) 5 days post-termination of drug. The benzodiazepine, diazepam (1.25-5 mg/kg), but neither the tricyclic antidepressent, imipramine (5- 20 mg/kg), the serotonin antagonist, buspirone (2.5-10 mg/kg) nor the dopamine receptor agonist, bromocryptine (10-40 mg/kg), were effective in blocking the PTZ-lever selection. These data suggest that there is an anxiety-like component in the cocaine withdrawal stimulus which may be mediated by a GABA-deficit and blocked by benzodiazepines such as diazepam. These data also demonstrate the utility of the drug discrimination methodology for investigating drugs of abuse. (Funded by the **American Osteopathic Association**).

388.16

EEG AND SUBJECTIVE RESPONSES TO INTRAVENOUS COCAINE IN HUMAN SUBSTANCE ABUSERS. M. Sano, N. Cascella*, B. Glover*, R. Herning* and E. London. NIDA Addiction Res. Ctr., Baltimore, MD 21224.

Cocaine (C)-induced increases in EEG β power have been noted (Berger, 1937; Herning, et al., 1985), but the time course of EEG changes and their potential relation to subjective effects have not been studied. We initiated a double-blind, placebo (P)-controlled, crossover study of C effects on subjective and EEG responses. Subjects were right-handed polydrug abusers (23-38 yr). Responses to 40 mg C or P i.v. were recorded from 13 scalp electrodes on the dominant hemisphere. EEG spectral power for α (10 Hz) and β (25 Hz) were calculated for 30 min after C or P. Subjective effects were recorded on a 5-point scale, using a 23-item questionnaire (Kumor et al., in press). Frontal and temporal lobes showed the greatest increases in β power; α power increased mostly in the occipital lobe. C effects on frontal and temporal β power and occipital α power occurred immediately. Mean occipital α power was elevated (vs. P) for 15-18 min after C. The frontal lobe β effect lasted up to 21-24 min, but the temporal lobe β effect persisted at 30 min. Self-reports of feeling "drug", "high" and "rush" were greatest at 1 min after C, with reductions by 15 min. Reports of feeling "good" also were greatest at 1 min, but persisted for 30 min. That C effects on temporal β power and feeling "good" persisted over approximately the same time suggests that the two phenomena may be related.

388.17

STRUCTURE ACTIVITY RELATIONSHIP OF COCAINE AND RELATED COMPOUNDS IN BINDING TO DOPAMINE TRANSPORTERS. M.C. Ritz*, E.J. Cone*, J. Sharkey* and M.J. Kuhar (SPON: R.K. Dismukes). NIDA Addiction Research Center, Baltimore, MD 21224.

The binding of cocaine to dopamine transporters appears to be a primary site of action related to the reinforcing properties of cocaine. We determined the potencies of a series of cocaine derivatives to inhibit ^{3}H-mazindol binding in order to determine the molecular requirements for their interaction at this site. The affinities of these compounds were substantially diminished by 1) isomerization to d-enantiomers; 2) epimerization of the tropane carbon C-2 substituents; and 3) hydrolysis of the C-2 or C-3 substituents to more polar forms. Moderate reductions in affinity resulted from 1) quaternization of the nitrogen; 2) replacement of C-2 substituent with hydrogen, and 3) methylation of the C-3 aromatic ring. Monocyclic and linear chain derivatives of cocaine incorporating the nitrogen and C-3 aromatic substituent also exhibited only moderate decreases in affinity. Modifications which increased affinity or resulted in little change were 1) replacement of the C-3 substituent with benzene or fluorobenzene or 2) N-demethylation. In summary, binding of cocaine derivatives to the mazindol site on the dopamine transporter appears to require the 1-isomeric form, including the nitrogen and C-3 aromatic substituents.

388.18

NEUROBEHAVIORAL EFFECTS OF PRENATAL COCAINE EXPOSURE. S.K. Sobrian, L.E. Burton*, N.L. Robinson*, H. James* and L.M. Turner*. Dept. of Pharmacol. HUCM, Washington, DC 20059.

Neurobehavioral ontogeny was studied in the offspring of pregnant Sprague-Dawley rats injected with 20 mg/kg of cocaine (s.c.) once daily on gestation days 15-21. This dose produced blood levels of 0.6 mg/ml of cocaine in the mothers; levels in the pups were not detectable at birth. Control mothers received injections of 0.9% NaCl (0.1 ml/100G). Birth statistics reflected the lack of maternal toxicity; third week gestational weight gains, and length of gestation were unaffected by maternal cocaine administration, as were little size, birth weights and lengths of pups and the male/female pup ratio. Although cocaine mothers were slower to initiate nest building, other indices of maternal behavior were unaltered. Cocaine exposed pups were significantly smaller than control offspring during the first 1.5 postnatal week; subsequent differences in body weights were not evident during the preweaning period. Although the appearance of physical landmarks was not affected by prenatal cocaine, delayed development of surface righting, cliff avoidance and startle response was noted. In utero cocaine exposure did not alter the offsprings response to postnatal stimulants; both cocaine (10 mg/kg) and amphetamine (1 mg/kg) increased spontaneous motor activity. These results indicate that in the absence of overt teratogenicity, fetal exposure to cocaine can delay neurobehavioral development.

388.19

PRENATAL EXPOSURE TO COCAINE IN RATS: EFFECTS ON LOCOMOTION AND STEREOTYPY. C.A. Moody, M. Giordano, E.M. Zubricki, L. Dreshfield*, R.A. Frank, A.B. Norman and P.R. Sanberg. Lab of Behavioral Neuroscience, Depts. of Psychiatry, Psychology, Neurosurgery and Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Pregnant Sprague-Dawley rats received s.c. injections of 30 mg/kg cocaine HCl or vehicle from day 12 to day 21 of gestation. Pinna detachment, eye opening, righting reflex, negative geotaxis, pivoting, startle response, body length and weight were recorded. Sixty days after birth the pups were monitored for spontaneous nocturnal activity, and drug-induced stereotypy. The stereotypic response to apomorphine (0,0.25, 1.0 mg/kg i.p.); amphetamine (0, 4.0, 8.0 mg/kg i.p.) and cocaine (0, 30 mg/kg i.p.) all at a volume of 1 ml/kg was visually evaluated using the Creese and Iversen (1973) rating scale.

All pups survived throughout the length of the study. Developmental cues appeared at the same rate in all animals regardless of the group to which they belonged. Both groups responded similarly to the psychomotor stimulants as compared to each group's own control session.

This pilot study suggests that prenatal exposure to a mild dose of cocaine does not have deleterious effects on the appearance of developmental cues in rat pups. In addition there was no apparent increase or decrease of sensitivity in the dopaminergic system as evidenced by the lack of differential response to dopaminergic agonists.

389.1

THE EFFECTS OF AMPHETAMINE ON FOOD INTAKE AND BODY WEIGHT IN RATS ALLOWED 24-HR ACCESS TO FOOD. J.R. Jones* & W.F. Caul. Department of Psychololgy Vanderbilt University, Nashville, TN 37240.

Recent investigations of tolerance to amphetamine's anorectic effect (Caul, et al., Behav. Neurosci., 102:441-450, 1988) have suggested the importance of assessing both within-day and day-to-day body weight changes as well as food intake of rats allowed continuous access to food in order to understand the role of homeostatic adaptive mechanisms. In this experiment, independent groups of rats maintained with a LD 12:12 light cycle were injected for 10 consecutive days with either 0, 1, or 2 mg/kg d-amphetamine sulfate either one hour after the lights came on each day or immediately before the lights were turned off. The results showed that "Daytime" injections produced no effects on daily food consumption, however, the drug-treated rats lost weight over days. "Nighttime" drug injections produced increased daily consumption yet day-to-day body weights were comparable to controls. Further analysis of intake and body weight in terms of two 12-hr periods each day, provides a better understanding of amphetamine's effect on eating, homeostatic adaptive mechanisms, and how these factors interact with the circadian organization of eating.

389.2

THE EFFECT OF DEPRIVATION LEVEL ON THE DEVELOPMENT OF TOLERANCE TO AMPHETAMINE ANOREXIA: BEHAVIOURAL AND NEUROCHEMICAL PERSPECTIVES. A. Streather, A.E. Le*, R.E. Hinson, C.X. Poulos*, and H. Cappell*. University of Western Ontario, London, Ontario; Addiction Research Foundation and University of Toronto, Toronto, Ontario, Canada.

The effect of deprivation level on the development of tolerance to amphetamine anorexia was investigated. The neurochemical consequences of tolerance development were also examined. Seventy-three rats, sensitive to amphetamine (AMP) anorexia, were divided into 6 groups. Three groups were maintained at 85% *ad lib.* weight while 3 groups had free access to food (100% groups). Three training groups, a contingent (CONT), noncontingent (NONCON) and saline (SAL), each comprised a 100% group and an 85% group. The CONT groups received 3 mg/kg AMP ip 20 min before 30 min access to milk on Day 1 and saline ip on Day 2. The NONCON groups received saline ip 20 min before 30 min access to milk on Day 1 and 3 mg/kg AMP ip on Day 2. The SAL groups received saline ip 20 min before 30 min access to milk on Day 1 and saline ip on Day 2. After 20 sessions the 100% groups were reduced to 85% *ad lib.* weight before an AMP anorexia test was given. The CONT 85% group consumed significantly more than any other group while the CONT 100% group showed no tolerance to AMP anorexia. At the time of the AMP anorexia test half of the 85% groups were sacrificed 20 min after injection of 3 mg/kg AMP. Later NE, DA, 5-HT, DOPAC, HVA, & 5-HIAA levels were assayed in several brain areas. Preliminary analysis revealed no central differences between the CONT and NONCON groups. The failure of the 100% CONT group to develop tolerance demonstrates that food deprivation is essential to the development of tolerance to amphetamine anorexia.

389.3

PERIPHERALLY OR CENTRALLY ADMINISTERED D-AMPHETAMINE INCREASES THE INTAKE OF CHOW SWEETENED WITH SUGAR BUT NOT SACCHARIN. Evans, K.R. and Vaccarino, F.J. Dept. of Psychology, University of Toronto, Canada.

We have previously demonstrated that when faced with a choice of different food types, low doses of peripherally or intra-nucleus accumbens (N.Acc.) administered d-amphetamine (AMP) preferentially increases the intake of palatable foods such as sucrose. The present study examined whether this AMP-induced/sucrose-selective increase in food intake was related to sweetness or some post-ingestional effect of sucrose. Rats were presented with powdered chow and chow sweetened to an equal degree with either sucrose or saccharin following treatments with systemically administered AMP (0.25 mg/Kg) or intra-N.Acc. AMP (0.5 ug or 2.0 ug). Baseline food intake did not differ in the sweetened chow conditions. AMP significantly increased intake of chow sweetened with sugar but had no effect on intake of unsweetened chow or chow sweetened with saccharin. Results suggest post-ingestional factors may be important with respect to AMP-induced feeding. Alternatively, AMP may cause animals to be more sensitive to any aversive properties saccharin might have. Further, the nucleus accumbens supports these effects, consistent with the view that this site is critical in the expression of the facilitatory effect of AMP on feeding.

389.4

SERTRALINE, A 5HT UPTAKE INHIBITOR, INHIBITS FEEDING AND BODY WEIGHT GAIN IN RODENTS. J. A. Nielsen and M. N. Krupp*. Pfizer Central Research, Depts. Neuroscience and General Pharmacology, Groton, CT 06340.

An extensive literature implicates brain serotonin in the regulation of energy balance (Blundell, Neuropharmacology 23:1537, 1984). More recently, attention has been focused on the ability of serotonin uptake inhibitors to reduce body weight both in laboratory animals and obese humans. Sertraline is a potent and highly selective inhibitor of serotonin uptake (Koe et al., J. Pharmacol. Exp. Ther. 226:686, 1983) and we have conducted preliminary studies to assess its effect on feeding and body weight in rodents.

Sertraline decreased food intake up to 70% and body weight up to 28% in normal mice without any effect on locomotor behavior. Similar effects were observed in normal rats, genetically obese ob/ob mice and fa/fa rats. These effects developed rapidly after initiation of sertraline treatment and were maintained during 5-7 days of continued drug administration. Nonspecific disruption of behavior does not account for the observed effects on feeding and body weight since the sertraline treated animals appeared healthy and their locomotor behavior was unaffected.

Thus, these results are in accord with a growing body of evidence suggesting that selective serotonin uptake inhibitors may be clinically useful agents for managing some obese patient populations.

389.5

WEIGHT LOSS EFFECT OF FLUOXETINE IN NORMAL-WEIGHT AND OVER-WEIGHT BULIMICS. J.M. Jonas,* M.S. Gold, L. Bunte,* A.L.C. Pottash. Fair Oaks Hospital, Summit, NJ 07901.

Fluoxetine is a relatively specific serotonin uptake inhibitor which may produce weight loss. In order to investigate this phenomena, we compared the effect of fluoxetine on weight and eating symptoms in 2 groups of bulimic individuals-a normal weight group (N=11) and a group (N=5) of individuals greater than 10% above ideal body weight. All patients met DSM-III-R criteria for bulimia nervosa, were treated a minimum of 8 weeks, were on no structured meal program, and received a minimum of 40 mg of fluoxetine each day. Response was rated in terms of decreased binging and purging, and weight change. Nine of the 16 subjects attained at least a 75% reduction in symptoms. The overweight group was more likely to show improvement in symptoms, with all 5 attaining at least a 75% reduction in symptoms, while among the normal weight bulimics, 4 of 11 (36%) displayed similar improvement (X^2=8.785; $p<.04$). Fluoxetine appeared to have a modest weight loss effect on the group as a whole, with the mean weight decreasing from 144.5 pounds (SD=38.6) to 141.8 pounds (SD=38.8) ($p<.07$ by paired t-test, two tailed). We expected that there would be a relationship between weight loss and decreased binging and purging, but this was not observed. Weight change was not related to outcome as determined by decreased binging (ANOVA p=.83), nor did it differ between groups (t-test for independent samples two-tailed, $p<.53$). The implications of these findings will be discussed.

389.6

FOREBRAIN STRUCTURES ASSOCIATED WITH DEPRIVATION INDUCED THIRST IN THE PIGEON: A (14C) 2-DEOXYGLUCOSE STUDY. K.P. Heuston. Department of Psychology and Social Relations, Harvard University, Cambridge, MA 02138.

(14C) 2-deoxyglucose (2-DG) was used as a metabolic tracer to identify forebrain structures associated with deprivation induced thirst in the pigeon. Pigeons were either water deprived, or else given free access to water, then injected with 2-DG (160uCi/Kg IV). The animals were sacrified, and their brains were removed, sectioned, and processed for autoradiography.

Autoradiographic images were analyzed, and, as compared to the control group, the water deprived animals showed increased 2-DG uptake in a number of telencephalic and diencephalic structures and areas, including a cluster of cells apposing (both ventrally and dorsally) the pallial commissure (a region which houses the subseptal (subfornical) organ in the pigeon).

Reconstructed autoradiographic images for a series of forebrain sections will also be presented.

389.7

CHRONIC D-FENFLURAMINE PRETREATMENT FAILS TO AFFECT SEROTONIN RELEASE FROM RAT HYPOTHALAMIC SLICES. J.D. Schaechter*, E. Armstrong* and R.J. Wurtman. (SPON: S.J. Schein). Lab. of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139.

D-fenfluramine produces anorexia in rats and man probably by raising intrasynaptic levels of serotonin (5-HT) in the brain via inhibiting its reuptake and promoting its release. A single low dose of d-fenfluramine reduces food intake (ED_{50}=1.3mg/kg). Repeated administration of a high dose of d-fenfluramine (≥10mg/kg) produces a long-lasting depletion (~50%) of brain 5-HT and its metabolite, 5-HIAA; though lower doses of the d-enantiomer (<5mg/kg) have produced no depletion of brain 5-HT. We have applied a novel in vitro experimental system, utilizing superfused rat hypothalamic slices, to address whether endogenous 5-HT release is altered following chronic d-fenfluramine treatment. Rats were treated for 10 days with d-fenfluramine (1.25; 2.5; 5; or 10 mg/kg/day; i.p.). Hypothalamic slices were prepared on day 16. The amounts of 5-HT (and 5-HIAA) released under basal conditions and during electrical field-stimulation (5Hz, 2ms, 100mA/cm^2, 1400 bipolar pulses) were monitored. Hypothalamic 5-hydroxyindole contents were unchanged in rats treated with 1.25 and 2.5 mg/kg/day of d-fenfluramine. Rats which received 5 and 10 mg/kg/day had lowered hypothalamic 5-HT levels (by 18% and 22%, respectively) and lowered 5-HIAA levels (by 20% and 29%). Chronic d-fenfluramine treatment did not affect basal 5-HT release at any drug dose tested. The evoked release of 5-HT and the efflux of 5-HIAA were unaltered from those slices prepared from rats treated with 1.25-5 mg/kg/day of the drug, and reduced only with the highest dose (by 20% and 37%, respectively). These results suggest that chronic administration of a low anorectic dose of d-fenfluramine (1.25-2.5 mg/kg/day) does not produce long-lasting changes in hypothalamic 5-hydroxyindole content nor alter the amount of 5-HT released spontaneously and with neuronal firing.

389.8

REGIONAL BRAIN SEROTONIN (5-HT) FOLLOWING FREE CHOICE INTAKE. J.L. Colmenares. Centro de Investigaciones Biomédicas, UNEFM., Coro, VENEZUELA.

Male Sprague-Dawley rats were fed for 5 days (Pretreatment) one of the following isocaloric diets: 18% Casein hydrolyzate (Tryptophan-free, TF); 18% Casein (Control, C) or Carbohydrate-fat (Protein-free, PF). Brain of rats TF-pretreated had 5-HT levels decreased to 80-75% of control values in the Cortex, Brain Stem, Hypothalamus and Cerebellum ($p<0.05$). Rats PF-pretreated showed 5-HT increases only in the cerebral cortex (122% of control values, $p<0.08$). Other set of rats were allowed to consume 5 or 45% casein diets presented simultaneously for the next 24 hours after their initial dietary pretreatment. 5-HT levels became equal in the regions analyzed, with the exception of the Hypothalamus of the PF-pretreated group, whose levels of 5-HT and 5-HIAA where increased to 127 and 130% of C values ($p<0.07$). The intake of the 5 and 45% protein diets by pretreated rats was significantly different (ANOVA, $p < 0.05$). During the free choice period the TF-pretreated ate respectively 117 and 207% calories of the C- and PF-pretreated groups. The PF-pretreated consumed respectively 115 and 200% of the protein of the TF- and C-pretreated groups. 5-HT cortical changes may participate in the craving for protein and total caloric intake. Hypothalamic 5-HT levels paradoxically increased only after high protein intake. (Supported by Fundacite No F1.22.01.83).

389.9

INTRANIGRAL INJECTION OF NEUROTENSIN SUPPRESSES FEEDING IN FOOD-DEPRIVED RATS. T.G. Anticich*, A.A. Baumeister, A. M. Vaughn*, and M.F. Hawkins. Department of Psychology, Louisiana State University, Baton Rouge, LA 70803.

Intraventricular injection of neurotensin (NT) suppresses feeding in food-deprived rats (Luttinger et al., Eur. J. Pharmacol, 81, 1982, 499). The neuroanatomical loci that mediate this effect have not been well defined. The substantia nigra (SN) is involved in ingestive behavior (Ungerstedt, Acta Physiol. Scand., 1971, Suppl. 367, 95) and contains high levels of NT receptors (Young et al., Brain Res.,206, 1981, 273). The present study was conducted to determine whether NT receptors in the SN may be involved in feeding. Male Sprague Dawley rats were deprived of food for 18 hours prior to receiving a bilateral intranigral injection of NT (2.5, 5.0, or 10.0µg/ 0.5µl saline) or saline (0.5µl). Food consumption was measured at 15 minute intervals for 2 hours after injection. Neurotensin suppressed food intake ($p<.05$) during the first 15 minutes at the high and low doses but not at the intermediate dose. During the second 15 minute interval only the high dose of NT suppressed feeding. No effects on feeding were observed after 30 minutes. Intranigral injection of NT at these doses produced no behavioral stereotypies or other signs of general motor impairment. These results suggest that the SN may mediate the hypophagic effect of NT. (Supported by USPHS grant HD-21560).

389.10

FEEDING RESPONSE OF THE SPONTANEOULSY DIABETIC RAT (SDR) TO NOVELTY-INDUCED STRESS AND SCH 23390. Q.Ahmad[1] and Z. Merali[1,2]. [1]Psychology and [2]Pharmacology, Univ. of Ottawa, Ontario, K1N 9A9.

This study assessed the feeding response of insulin treated male SDR and matched controls (n=6 each) to: 1) SCH 23390 (SCH) a D-1 receptor antagonist, and 2) novelty stress (NOV). All animals were entrained on a regimen of 17.5 hrs of food deprivation, followed by treatment, presentation of test meal, and behavioral monitoring (for 30 min). In the SDR, SCH (10, 40 ug/kg) incurred a 62% and 27% increase in meal size respectively, as compared to a 3% and 29% increase in controls. Conversely, SCH (100 ug/kg) reduced the meal size by 72% and 30% in the SDR and controls respectively. NOV increased the meal size of SDR and controls by 42% and 24% respectively. Interestingly, at low doses SCH elicits grooming but blocks it at high doses. A similar inverted U dose-response was seen here, with low doses of SCH stimulating and high doses inhibiting the feeding response. We reported earlier that the SDR displays an increased sensitivity to SKF 38393 and novelty stress-induced grooming. In this case, the dose-response curve of the SDR appears to have shifted to the left, again implicating increased sensitivity of the SDR to D-1 receptor and stress associated responses.

389.11

Differential effect of dopamine D-1 and D-2 receptor antagonists on the microstructure of ingestive behavior. J.D. Davis, J.W. Kebabian & C. Vasilatos*, Dept. of Psychology, Univ. of Illinois, Chicago, IL 60680 & Neuroscience Res. Div. Abbott Laboratories, Abbott Park , IL 60064.

Rats ingest liquid diets by licking a drinking tube in bursts separated by pauses. This study determined the effect of selective dopamine D-1 and D-2 receptor antagonism on the size of bursts (SB) and the length of the interburst interval (IBI). Male albino Sprague Dawley rats were trained to drink a highly palatable test diet (.006M Saccharin + 0.1M maltose) for 30 min a day. Time of tongue contact with the drinking tube was measured to the nearest 10 msec by an electronic drinkometer. SCH23390, a selective dopamine D-1 receptor antagonist at doses of .012 to .015 mg/kg (s.q.) reduced volumetric intake in a dose related manner by increasing the IBI and the number of bursts sufficiently to compensate for a significant increase in SB. Piquindone, a selective dopamine D-2 receptor at doses from .03 to .16 mg/kg reduced volumetric intake in a dose related manner principally by decreasing the size of the burst. IBI and number of bursts were significantly altered (increased and decreased respectively) only at the highest dose. The inactive enantiomers of both compounds were ineffective in altering volume intake or the two parameters. We conclude that antagonism of the dopamine D-1 receptor has a different effect on ingestive behavior than does D-2 receptor antagonism.

389.12

RATS SHAM FEEDING CORN OIL ARE LESS SENSITIVE TO DOPAMINE RECEPTOR ANTAGONISTS THAN RATS SHAM FEEDING SUCROSE. G.P. Smith and S.C. Weatherford. Dept. Psychiatry, NY Hosp.-Cornell Med Ctr., White Plains NY 10605.

Central dopaminergic (DA) systems are thought to participate in the reward aspects of sham feeding (SF) sucrose (SUC) and corn oil (CO) in rats. Here we examine the effect of the selective D-1 and D-2 receptor antagonists, SCH 23390 (SCH) and raclopride (RAC), respectively, on the intake of 100% CO and 10% SUC in SF rats. RESULTS Values are mean % inhibition of sham intake ± SEM.

RAC	SUC	CO	SCH	SUC	CO
.050	15±8	--	.0125	11±5	--
.100	40±10*#	-8±9	.025	29±11*#	2±5
.200	80±7*#	40±10*	.050	58±10*#	10±10
.300	92±3*	59±10*	.100	80±6*	51±12*
.400	97±7*	80±7*	.200	92±4*	93±3*

Note: tests were conducted in the a.m. after 18 h food deprivation, N=12, doses are mg/kg,i.p., given 15 min before SUC or CO. *drug vs veh, #CO vs SUC, $p < .01$

CONCLUSION When SF CO, rats are less sensitive to D-1 and D-2 antagonists than when SF SUC. It is not clear whether this difference in sensitivity is due to a greater release of DA during CO SF than during SUC SF, or to the possibility that SF CO may be less dependent on DA systems than the SF of SUC. Neurochemical studies are needed to distinquish between these possibilities. [Supported by MH15455 (GPS).]

389.13

SHAM FEEDING RATS PREFER CORN OIL TO SUCROSE, BUT WHEN REAL FEEDING, THE PREFERENCE IS REVERSED.

SC Weatherford, GP Smith and J Gibbs. Dept. of Psychiatry N.Y. Hosp.-Cornell Med. Ctr, White Plains NY 10605.

Rats sham feed (SF) corn oil (CO) and sucrose (SUC) in a concentration-dependent manner, suggesting that the orosensory stimuli of these solutions are positively reinforcing. The purpose of this study was to determine the rank order of preference of 100% CO, 6% SUC and 10% SUC in the absence (SFing; cannula open) and presence (real feeding; cannula closed) of postingestive cues.

Three groups of overnight food-deprived rats were trained to SF 2 solutions in alternate 1-bottle tests. Groups 1,2 and 3 sham fed: 100% CO and 6% SUC; 100% CO and 10% SUC; and 10% SUC and 6% SUC, respectively. Rats then received two 30-min, 2-bottle preference tests. Following the SFing preference tests, rats were given three 30-min, 2-bottle preference tests with the cannula closed.

During SFing the rank order of preference was 100% CO >> 10% SUC >> 6% SUC. In contrast, when the cannula was closed, the same order of preference was observed in the first preference test, but by the third preference test the order had changed to 10% SUC >> 6% SUC >> 100% CO.

Thus,in the absence of postingestive cues the orosensory stimulation of CO is more reinforcing than SUC. When postingestive cues are present, however, CO becomes less reinforcing than SUC, while the preference for 10% SUC over 6% SUC remains unchanged.[Supported by MH15455 (GPS).]

389.14

DENSITY OF α_1- AND α_2-NORADRENERGIC RECEPTORS IN THE DISCRETE HYPOTHALAMIC AND EXTRA-HYPOTHALAMIC AREAS OF GENETICALLY OBESE ZUCKER RATS.

J. A. Finkelstein, S. R. Kim*, I. Awad*, S. F. Leibowitz, M. Jhanwar-Uniyal.(SPON: N. Miller). N. E. Ohio Univ., Col. Med. Rootstown Ohio 44272, The Rockefeller Univ. New York, N.Y. 10021.

Administration of norepinephrine (NE) into the paraventricular nucleus (PVN) produces eating via the activation of α_2-noradrenergic receptors. Studies suggest that NE levels differ in certain hypothalamic nuclei (including PVN) of Zucker obese vs. lean rats, and therefore may contribute to the development of obesity. The present study examines the density of α_1-and α_2-noradrenergic receptors in discrete hypothalamic and extra-hypothalamic areas of lean and genetically obese rats.

Female, lean (Fa/-) and obese (fa/fa) Zucker rats were used for this study. Seven hypothalamic nuclei, namely, PVN, medial preoptic nucleus, dorsomedial nucleus, ventromedial nucleus, perifornical lateral hypothalamus, supraoptic nucleus and arcuate nucleus-median eminence and four extra-hypothalamic sites (caudate nucleus, nucleus accumbens, hippocampus and frontal cortex) were micropunched. Standard radioligand binding procedures, using the α_2-noradrenergic agonist, [3H]p-aminoclonidine ([3H]PAC; 2.7 nM) and the α_1-noradrenergic antagonist [3H]prazosin (1.0 nM) were employed. Nonspecific binding was determined in the presence of phentolamine (50 µM). The results demonstrate that genetically obese, as compared to lean, littermates had: a) an increase in [3H]PAC binding in the PVN (152%) and caudate nucleus ($p<0.05$; 317%); and b) an increment in [3H]prazosin binding only in the PVN ($p<0.05$; 250%). These findings suggest a possible role of α-noradrenergic receptors in the development or maintenance of genetic obesity.

389.15

OCTOPAMINE INJECTED INTO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVN) ELICITS FEEDING IN SATIATED RATS.

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The trace amine octopamine (OA), which occurs naturally in mammalian brain, has been reported to possess significant actions on noradrenergic systems. The present experiments were designed to examine the effects of the meta and para isomers of OA on feeding behaviour in satiated rats following injection into the PVN, and to compare them with those elicited by noradrenaline (NA).

m-OA induced a dose-dependent increase in food intake which was maximal at 25 nmoles/0.4 µl injection. *p*-OA did not increase food intake unless the rats were pretreated with the monoamine oxidase inhibitor pargyline. The effect of *m*-OA was blocked by the α2-antagonists yohimbine (12.5 and 25 nmoles) and idazoxan (25 and 50 nmoles), but not by the α1-antagonist corynanthine (25 and 50 nmoles), or the β-antagonist propranolol (50 and 100 nmoles). Feeding elicited by 25 nmoles NA was also reversed by idazoxan (but not by yohimbine), but was not affected by corynanthine or propranolol. Depletion of endogenous NA stores by α-methyl-*p*-tyrosine (200 nmoles injected into the PVN, 4 hours) partially blocked the effect of *m*-OA. Inhibition of the NA reuptake system with desipramine (100 nmoles injected into the PVN) significantly enhanced the effect of 12.5 nmoles *m*-OA, but not of 25 nmoles *m*-OA.

The results show that *m*-OA injected into the PVN elicits feeding. This effect occurs via an action at α2 receptors, and appears to involve, at least partially, the release of endogenous NA.

P.J.F. and I.A.P. are Saskatchewan Health Research Board Fellows.

389.16

EFFECTS OF ADRENALECTOMY AND HORMONE REPLACEMENT ON NATURAL FEEDING PATTERNS IN THE RAT.

D.L. Tempel, M. Bauer* & S.F. Leibowitz Rockefeller University New York, N.Y. 10021.

Patterns of food intake and macronutrient selection are known to vary as a function of time of the diurnal cycle. When maintained on pure diets of protein (P), carbohydrate (C) and fat (F), freely-feeding animals display a natural preference for the C diet at dark onset, and for the P and F diets at the end of the night. The enhanced carbohydrate seen at dark onset is believed to be mediated by norepinephrine (NE), particularly in the hypothalamic paraventricular nucleus (PVN), in concert with circulating adrenal hormones, specifically corticosterone (CORT). In support of this hypothesis, we have found that adrenalectomy (ADX) abolishes the natural carbohydrate feeding at dark onset (from 3.3 Kcal in SHAM to 0.3 Kcal in ADX animals $p<0.05$). Direct PVN CORT implant reinstates C feeding at this time (to 4.2 Kcal, $p<0.05$). PVN implant of the mineralocorticoid aldosterone (ALDO) also stimulates feeding of C (4 Kcal), as well as F (5 Kcal), during hr 1 of the dark phase. Similar affects of ALDO were seen in SHAM animals. Deoxycorticosterone (DOC) has no effect and dexamethasone (DEX) is less effective ($p<0.05$) than either CORT or ALDO in enhancing feeding at this time of the cycle. In contrast to its effects on food intake at dark onset, ADX has no effect on natural feeding during hr 12 of the dark cycle. Similarly, CORT, DOC and DEX are ineffective in stimulating food intake at this time. However, PVN ALDO implant enhances F and to a lesser extent, C intake during hr 12 of the dark phase in both ADX and SHAM rats. These experiments will help elucidate the neuroendocrine mechanisms, and possible glucocorticoid receptor type underlying natural feeding processes.

389.17

EFFECTS OF CLONIDINE ON SEPARATE SELF-SELECTION OF MACRO AND MICRONUTRIENTS.

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The following experiment studied the effects of Clonidine, (CLON) an alpha adrenergic agonist, on the consumption of macronutrients (fats, proteins and carbohydrates) and vitamins (A,D,E,B complex) and minerals (calcium, sodium chloride, potassium chloride, sodium phosphate) in the adult male rat. The self-selection feeding paradigm was fashioned after Richter et al (1938), who found that various physiological manipulations affected the intakes of both macro- and micronutrients. Although Leibowitz et al (1984) was able to determine the effects of CLON on self-selection of macronutrients, no information is available about CLON's effects on separate selection of macro- and micronutrients. Because Richter et al (1938) showed the importance of separate intakes of macro- and micronutrients, it was important to extend the Leibowitz et al (1984) findings to the effects of CLON.

All nutrients were available simultaneously and were eaten ad lib. Baseline measurements were taken five days prior to IP drug injections of 25 ug/kg of CLON, which was found by Mauron et al (1980) and Leibowitz et al (1984) to be the most effect dose for eliciting consumption of various macronutrients. CLON was injected 1-2 hours after onset of darkness, which was the optimal feeding time noted by Leibowitz et al (1984). Measurement of the nutrients were made on a 1 hour and a 12 hour period after injections. Testing was done under natural lighting conditions. We found a significant increase in carbohydrate consumption, but no effects on the consumption of other nutrients. The increase in carbohydrates is in accordance with Leibowitz et al (1984) findings, but differ from those of Mauron et al (1980) who found enlarged protein intake. Since no attempt was made to test weanling animals, no information on differences in selection as observed by Mauron et al (1980) is available. In conclusion, CLON enhances intake of carbohydrates, but has no influence on intake of other macronutrients or micronutrients when they are offered separately.

389.18

α-NORADRENERGIC EFFECTS ON MACRONUTRIENT SELECTION IN GENETICALLY OBESE AND LEAN MICE.

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Although α_2-NA mechanisms increase food intake, especially carbohydrate(CHO) in rats(Leibowitz et al.,Pharm.Biochem. Behav.,23:541,1985), Callahan et al.(Pharm.Biochem.Behav., 20:591,1984) showed that both the α_2 agonist clonidine(CLON) and the α_2 blocker yohimbine HCl(YOH) decreased total intake (g) in obese(ob) and lean(+/?) mice, with obs showing a biphasic response to CLON and suppressing intakes at lower YOH doses than leans. STUDY 1: Obs and leans adapted to 6-h access to CHO, FAT, and protein(PRO) before receiving saline(VEH) injections ip 30 min before diet access, for 2 days, with intakes recorded at 1,3, and 6h. Then, separate groups(n=14/group) of obs and leans got either .1 or .5mg/kg BW CLON(Sigma) or VEH, 30 min before diet access. CLON decreased total intake(kcal) and Kcal from CHO,FAT, and PRO in ob and lean($p<.005$). But, at 1h, .5-CLON increased the proportion of energy from CHO and PRO and decreased from FAT. STUDY 2: When mice had either VEH, or 3 or 5 mg/kg ip YOH 30 min before either .5-CLON or VEH(n=7/group), both YOH and CLON decreased total intake($p<.001$), although the CHO proportion was greater when 3-or 5-YOH preceded CLON, or 5-YOH preceded VEH, than when either 3-YOH or VEH preceded VEH or CLON, or VEH alone($p<.001$). All drug groups suppressed FAT proportion($p<.05$). Although CLON and YOH alone had similar effects, in some cases joint administration reversed the effect either drug had on its own. There were no Phenotype X Drug interactions. (Supported by NSERC-A7937)

390.1

DOPAMINE AND Ca++-BINDING PROTEIN-CONTAINING NEURONS IN THE DORSAL TIER OF THE SUBSTANTIA NIGRA PARS COMPACTA IN NEONATAL- AND ADULT-6-OHDA LESIONED RATS. **L.M. Grimes, M. Sar.*, W. Stumpf, G.R. Breese, H. Criswell, R.A. Mueller, J.-S. Hong, and H.-K. Jiang*. UNC Sch. Med., Chapel Hill, NC 27599 and NIEHS, NIH, RTP, NC 27709**

Rats with neonatal destruction of dopamine (DA)-containing neurons challenged with DA agonists display different behavioral responses than those seen when lesioning is in mature rats (JPET 231:343, 1984). Recent reports have demonstrated a 28 KDa Ca++-binding protein (CaBP) in dorsal tier mesostriatal DAergic neurons which are developmentally distinct (J. Neurosci. 7:3935, 1987). In the present investigation, immunohistochemistry of tyrosine hydroxylase (TH) or CaBP neurons was performed on rats treated neonatally or as adults with 6-OHDA. TH neurons in A-8 and in the A-9 cell groups of the ventral tier were absent in both adult- and neonatal-6-OHDA-lesioned rats. In contrast, TH and CaBP neurons in the dorsal tier were spared in adult-lesioned rats, but were absent in neonatal-lesioned rats that exhibited self-mutilation (SMB) when challenged with apomorphine. Sparing of DA and CaBP neurons in the dorsal tier was associated with sparing of terminals in the ventral striatum. Future studies will define the relationship between CaBP/TH-containing neurons and behavioral responses to DA agonists after 6-OHDA lesions. [Supported by ES-07125(LMG), HD-03110, NS-21345]

390.2

CHARACTERIZATION OF THE MOTOR AND SENSORIMOTOR FUNCTIONS OF BRAIN DOPAMINE J.D. Salamone, M.J. Zigmond, E.M. Stricker. (SPON: Shang Yao). Dept. of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

Dopamine (DA) antagonists or lesions of brain DA neurons produce acute decreases in motor activity and responsiveness to stimuli. To study the sensorimotor effects of haloperidol (HP), rats were tested for conditioned responses to auditory stimuli presented at varied intervals. In different experiments the intensity or duration of the stimulus was varied, and food-deprived rats were rewarded for responding during the stimulus. HP suppressed responding to high and low intensity stimuli equally. In contrast, the anticholinergic atropine had greater effects on the low intensity stimulus. HP also had a greater effect if the rats were required to respond within 2 rather than 5 sec. In a second series of experiments, food-deprived rats were tested in 30-min feeding sessions. As well as measuring food and water intake, an observer kept real-time event records of feeding, drinking and rearing. HP or 6-OHDA lesions decreased food and water intake, total time feeding, rate of feeding, average duration of periods of feeding, and rearing. Feeding rats before the session decreased food intake largely by suppressing feeding time and the number of feeding periods, with little effect on the rate of feeding, and no suppression of rearing. Taken together, these results indicate that HP and lesions of DA neurons decrease motor activity and responsiveness to stimuli in ways that do not resemble interference with sensory processes or a reduction of all aspects of food motivation.

390.3

PARADOXICAL KINESIA IN RATS WITH LARGE DOPAMINE (DA)-DEPLETING BRAIN LESIONS IS NOT MEDIATED BY DA. K. A. Keefe, J. D. Salamone, M. J. Zigmond, & E. M. Stricker, Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Rats become akinetic after large DA-depleting brain lesions, yet swim when placed in water and escape from a floating ice bath. Because such stressors increase DA release in intact animals, we examined the hypothesis that this paradoxical kinesia results from DA release from residual DA neurons. In the present studies, the effects of putative DA release were inhibited by treating brain-damaged rats with haloperidol. Rats were akinetic 2 days after near total 6-hydroxydopamine (6-HDA)-induced lesions of the nigrostriatal pathway. Nevertheless, when they were given haloperidol (1 mg/kg) and placed in 31-cm-deep water for 10 min, rats swam effectively for the entire test, although not as vigorously as controls. Enhanced motor function also was seen for a short period (<120 s) after the swim test. In contrast, the haloperidol completely blocked the behavioral response to amphetamine (5 mg/kg). Other 6-HDA-lesioned rats pretreated with haloperidol escaped from a floating ice bath in a 30-cm-deep tank, as they had done prior to brain damage. These results suggest that paradoxical kinesia is not a consequence of DA release from residual DA fibers.
(Supported by USPHS grants NS19608 and MH29670)

390.4

NEUROPHYSIOLOGICAL STUDIES OF SENSORY GATING IN RAT HIPPOCAMPUS: EFFECTS OF AMPHETAMINE AND HALOPERIDOL H.T. Nagamoto*, P.C. Bickford-Wimer, L.E. Adler, M. Egan*, R. G. Johnson*, R. Freedman and G.M. Rose. Depts. of Pharmacology and Psychiatry, VAMC and USHSC Denver, Colorado 80262.

Central mechanisms of sensory gating were assessed in Sprague-Dawley rats by an evoked potential technique similar to one that we have used to show diminished sensory gating in psychotic human subjects. In previous studies, auditory evoked potentials (AEPs) were recorded from the skull of unanesthetized freely moving rats using a conditioning-testing paradigm. In this paradigm, gating mechanisms were assessed by measuring the suppression of response to a 74 dB click test stimulus following an earlier identical conditioning stimulus at 0.5-second intervals. The rats showed significant suppression of the N50 response to the second auditory stimulus. Amphetamine significantly interfered with this suppression; haloperidol injected afterwards returned suppression toward normal pre-amphetamine values. AEPs were recorded from the CA3 region of the hippocampus of rats anesthetized with Chloral Hydrate. These animals had a N40 response that showed significant conditioning-testing suppression. As with the skull potential in freely moving unanesthetized animals, amphetamine interfered with this suppression and haloperidol returned suppression toward normal values. Supported by VAMRS and USPHS MH-38321.

390.5

STEREOSELECTIVE DISRUPTION OF SENSORIMOTOR GATING BY N-ETHYL-3,4-METHYLENEDIOXYAMPHETAMINE (MDEA) IN THE RAT. M.A. Geyer and R.S. Mansbach. Dept. of Psychiatry, UCSD Sch. of Med., La Jolla, CA 92093.

N-ethyl-3,4-methylenedioxyamphetamine (MDEA) is a derivative of methylenedioxymethamphetamine (MDMA), a drug with demonstrated abuse liability. MDEA and MDMA share a destructive action on serotonin neurons and appear to induce some similar behavioral effects. The relative importance of hallucinogen-like and amphetamine-like properties in the behavioral effects of these drugs is not clearly understood. The present study investigated effects of racemic MDEA and its stereoisomers on prepulse inhibition, a model of sensorimotor gating that is sensitive to disruption by amphetamine. Rats were subjected to 120 dB acoustic pulses, some of which were preceded at a 100 msec interval by a weak 80 dB prepulse stimulus. After saline, the prepulse induced an approximate 50% reduction in whole-body startle responses elicited by the pulse. Administration of racemic or (+)MDEA (0.3-10.0 mg/kg) induced a significant loss of prepulse inhibition, as indicated by drug-by-trial interactions and increased startle responses to stimuli preceded by prepulses. Racemic MDMA (0.3-10.0 mg/kg) produced similar effects. In contrast, (-)MDEA (0.3-10.0 mg/kg) did not significantly decrease prepulse inhibition. The implications of this stereoselective blockade of prepulse inhibition by MDEA are discussed.

390.6

PRESENTATION OF WATER TO DEPRIVED RATS INCREASES CATECHOLAMINE (CA) METABOLISM IN DISCRETE BRAIN REGIONS. David Jolly* and Lewis Seiden (SPON: Dwight Hand).Dept. Pharm/Phys. Sciences, Univ. of Chicago, Chicago, IL 60637.

Inhibition of aromatic-l-amino acid decarboxylase by NSD-1055, the activity of tyrosine hydroxylase (TH) causes accumulation of DOPA (dihydroxyphenylacetic acid) in rat brain. If CA metabolism is a steady state, TH activity is proportional to release. Thus CA effects of operant testing may be manifest in differential DOPA accumulation. Water deprived rats on a variable-interval (VI) schedule had higher striatal DOPA accumulations than did ad-lib, non-VI rats ($p<.05$). Deprived rats non-contingently presented water (RT) had greater DOPA accumulations than did deprived control rats (sham). Also, RT rats had higher DOPA accumulations than did ad-lib controls (quiet). This pattern of DOPA accumulation was observed in striatum ($p<.05$), nucleus accumbens ($p<.001$; quiet vs RT $p<.01$, quiet vs sham $p<.05$), and in amygdala ($p<.001$); quiet vs RT $p<.01$, quiet vs sham $p<.05$. Sham and RT rats had identical hypothalamic DOPA levels ($p<.05$; quiet vs RT $p<.05$, quiet vs sham $p<.05$). Differential brain DOPA accumulation also occurs without operant testing. Deprived rats periodically presented water had higher DOPA accumulations in striatum than did ad-lib rats. Apparently, increased brain CA metabolism occurs irrespective of operant schedule or response contingency. This research was supported by PHS MH-14274; RSA MH-10562 (L. Seiden), MH-11191.

390.7

DOPAMINE CONCENTRATION INCREASES IN THE CEREBRAL CORTEX AND BRAIN STEM OF THE HIBERNATING LITTLE BROWN BAT (MYOTIS LUCIFUGUS). W.A. Bauman*, H. Sershen*, C.A. DeSalvo* and A. Hashim* (SPON: B. Scharrer). Veterans Administration Medical Center, Bronx, NY and Center for Neurochemistry, Ward's Island, NY.

Hibernation (H) is an adaptive orchestrated physiologic response to harsh environmental conditions. The changes in various bioamines during this behavior may afford insight into the mechanism of H and temperature regulation in general. To determine the effect of H on the concentrations of dopamine (DA) and its metabolite DOPAC in the cerebral cortex (CC) and brain stem (BS), we sacrificed euthermic nonhibernating (NH) bats in October and H bats (core body temp 6-20°C) in April. The brain was carefully dissected into CC and BS and stored at -50°C until extraction and assay of the supernatants using HPLC with electrochemical detection. Results are expressed as mean ± SEM:

State	CC	BS
NH	15±2✱/3.5±0.4▪	0.78±0.06✱/0.43±0.04
H	35±3✱/5.9±0.5▪	1.38±0.13✱/0.49±0.05

All concentrations are expressed in ng/mg protein
✱p<0.001, ▪p<.01

Conclusions: (1) CC DA and DOPAC concentrations markedly increased during H, (2) BS DA increased during H, and (3) the increase in DA concentration in the brain may be a generalized finding during H.

390.8

ENRICHED AND IMPOVERISHED ENVIRONMENTS: EFFECTS ON THE TURNOVER RATES OF MONOAMINE NEUROTRANSMITTERS. M. J. Renner (Department of Psychology, University of Wisconsin, Oshkosh, WI 54901), C. L. Blank, & K. Freeman (Department of Chemistry, University of Oklahoma, Norman, OK 73019).

Previously, we reported data concerning tissue concentrations of monoamine transmitters and their metabolites in rats after enriched and impoverished housing experience (Renner, et al., Soc Neur Abs, 1986, 12, 1136). Those studies are extended here by examining turnover rates of these transmitters. Sprague-Dawley male rats (27 wt-match pair, 70 days) were randomly assigned to either an enriched condition (EC; group housing, 75x75x40 cm cage, several objects, rotated daily) or an impoverished condition (IC; solitary housing, small cage, no cagemates), for 30 days. Ss were injected with 200 mg/kg of the L-aromatic amino acid decarboxlyase inhibitor NSD-1015, held 30 min, and sacrificed by 800 msec of 10kW microwave irradiation to the head at 2.45 GHz (New Japan Radio NJE-2603). Brains were dissected into 11 sections and analyzed via HPLC-EC using a reversed phase column packed with 3 u particles (Lin., et al., J. Lig. Chromatog., 1984, 7(3), 509-538). IC significantly exceeded EC in hippocampal serotonin turnover rate (5-HTP buildup; p=.002). Dopamine turnover (Dopa buildup) was significantly different in only one of two replications (p = .04). In occipital cortex, the region of largest EC-IC anatomical differences, no significant differences were found. These findings are opposite the direction of other brain differences reported for EC-IC comparisons.

390.9

ASCORBATE INFUSIONS INTO THE NEOSTRIATUM MODULATE COMPONENTS OF THE BEHAVIORAL RESPONSE TO AMPHETAMINE AND HALOPERIDOL. L. K. White, M. A. Maurer*, E. A. Sidell*, M. E. Kraft*, C. Oh* and G. V. Rebec. Department of Psychology, Indiana University, Bloomington, IN 47405.

The neostriatum contains high levels of ascorbate (AA), a water soluble vitamin (Milbey et al., Neurosci. Lett., 28: 169, 1982). Systemic or intraventricular administration of AA attenuates the behavioral response to amphetamine and potentiates the effects of haloperidol (e.g., White et al., Psychopharm., 94: 284, 1988). Because the neostriatum plays a key role in the behavioral response to these drugs, AA may exert its antiamphetamine effects in this site. To test this hypothesis, we infused AA (2.0 ug/ul) or saline (0.9%) bilaterally into the neostriatum of rats at a rate of 0.4 ul/min and monitored the behavioral response to 1.0 mg/kg d-amphetamine administered alone or preceded by 0.025 mg/kg haloperidol. Infusions of AA into the neostriatum attenuate components of the behavioral response to amphetamine and enhance the antiamphetamine effects of haloperidol. Thus, AA appears to antagonize dopamine transmission in the neostriatum.

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390.10

LONG-TERM ASCORBATE TREATMENT: DIFFERENTIAL EFFECTS ON THE BEHAVIORAL RESPONSE TO ACUTE AND CHRONIC AMPHETAMINE. G.V. Rebec, A. Basse-Tomusk,& M. Lam, Dept. Psychol, Indiana Univ., Bloomington, IN 47405.

Systemic, intraventricular, or intrastriatal ascorbate (AA) attenuates the behavioral response to amphetamine (AMPH)(White et al., Psychopharmacology 94:284,1988). Since AA is actively taken up and retained in the CNS for prolonged periods of time (Hammarstrom, Acta Physiol. Scand. 70:3, 1966), chronic AA pretreatment could alter the behavioral response to a subsequent AMPH challenge. In addition, repeated AMPH injections deplete striatal AA levels (Kamata et al. Brain Res. 362:331, 1986) suggesting that concurrent AA administration also may alter the behavioral sensitization that accompanies chronic AMPH treatment. To test these hypotheses, rats received 5.0 mg/kg d-AMPH in the morning and 250 mg/kg AA or saline in the evening for 6 days. Separate groups of rats were pretrated for the same period with saline in the morning and saline or AA in the evening. On the 7th day, all animals were challenged with 5.0 mg/kg AMPH and the behavioral response rated by an independent observer.

Although concurrent pretreatment with AA failed to alter AMPH-induced behavioral sensitizaton, chronic AA did affect the acute response to AMPH. Thus, AA-pretreated rats showed significantly less sniffing and more oral behaviors in response to AMPH than controls.

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390.11

CAFFEINE-INDUCED CONDITIONED PLACE PREFERENCE AND AVERSION. N.T. Brockwell, R. Eikelboom, and R.J. Beninger. Dept. Psychology, Queen's University, Kingston, Canada.

Although caffeine may be the most widely used behaviorally active drug, few studies have illustrated its reinforcing properties. The place conditioning paradigm has been used to illustrate the reinforcing effects of many drugs of abuse, such as the dopamine (DA) agonists cocaine and amphetamine. Following several pairings of a drug injection with one side of a dual-chambered box, the undrugged animal displays a preference for the drug-paired side. The present study utilized place conditioning to assess the rewarding properties of caffeine in male Wistar rats. Results indicate that a high dose (30 mg/kg IP or SC) produced a significant place aversion, whereas lower doses (0.3-10.0 mg/kg IP) produced place preferences. Using an identical procedure, (+)-amphetamine (2.0 mg/kg IP) produced a significant place preference. These results suggest that doses of caffeine produce a biphasic effect on place conditioning, and are consistent with in vivo electrochemical evidence (Morgan, Dunn, & Vestal, Neurosci. Abstr. 13:253, 1987) which suggests that low caffeine doses increase and high doses decrease caudate DA release. (Funded by NSERC).

390.12

EVIDENCE FOR OPIOID-DOPAMINE LINK IN A NEW ANIMAL MODEL OF MANIA. W.Fratta, P.Fadda*, M.C.Martellotta*, G.L.Gessa* Department of Neurosciences Bernard B. Brodie, University of Cagliari, Italy.

Sleep deprivation (S.D.) often precedes the onset of mania. Sleep deprived rats show a stage of excitement before they fall asleep that has striking similarities with symptoms of mania, such as insomnia, hyperactivity, aggressiveness and hypersexuality. To verify if such a response might represent a valid model of mania, we studied the effect of different drugs on the excitement following 72 hrs S.D. in rats. We found that the duration of the excitement and EEG arousal was markedly shortened by chronic lithium, by haloperidol (0.2 mg/Kg^{-1}) and SCH 23390 (at the dose as low as 3 ug/Kg^{-1}) but was not influenced by (-) sulpiride (25 mg/Kg^{-1}).
Moreover, S.D. induced excitement was suppressed by Naloxone and markedly prolonged by morphine and other opioids. The results suggest that S.D. -induced "manic-like" behaviour is mediated by the activation of an opioid-dopaminergic link leading to D_1 receptor stimulation.

390.13

DOPAMINERGIC RECEPTORS IN THE ANTERIOR HYPOTHALAMUS FACILITATE FELINE AFFECTIVE DEFENSE BEHAVIOR. S. Sweidan*, H. Edinger and A. Siegel. Depts. of Neurosciences and Physiology, UMDNJ, Newark, NJ, 07103.

The medial preoptico-anterior hypothalamus (mPO-AH) has been shown to be the origin of the descending fibers mediating hypothalamically-elicited affective defense behavior (AD) in the cat. Since dopaminergic (DA) fibers supply this region, we investigated the contribution of DA mechanisms in the mPO-AH to DA facilitation of AD.

Electrical stimulation was used to elicit AD from the ventromedial hypothalamus. Cannula-electrodes, implanted in the mPO-AH, were used for microinjection of drugs into mPO-AH sites from which AD could also be elicited. The effects of microinjections were determined by examining the changes in current threshold for eliciting AD. Following preinjection response threshold determinations, apomorphine solutions containing 10, 50, 100, 200 or 500ng were microinjected (0.25uL) into the mPO-AH. Apomorphine injections significantly facilitated this response as response thresholds decreased in a dose-dependent manner. Pretreatment with a DA antagonist haloperidol (500 ng into mPO-AH) blocked the facilitatory effect of apomorphine. Vehicle injections had no significant effect upon AD.

The results suggest that DA-receptor-mediated mechanisms in the mPO-AH are involved, at least in part, in the DA facilitation of affective defense behavior.

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390.14

COMPARISON OF SOCIAL-ISOLATION AND FRONTAL CORTEX DOPAMINE DEPLETION ON CONDITIONED LOCOMOTOR ACTIVITY. G.H. Jones,* T.W. Robbins and C.A. Marsden.*[1] (SPON: M.E. McCourt) Dept. of Experimental Psychology, University of Cambridge, Cambridge, U.K. CB2 3EB and [1]Dept. of Physiology & Pharmacology, Queen's Medical Centre, Nottingham, U.K. NG7 2UH.

When food deprived rats were given daily exposures to photocell activity cages, those reared in isolation were hyperactive on the first two days of testing, when compared to socially-reared controls. By day 3 there was no difference between the two groups. However, when testing was paired with food presentation, the locomotor hyperactivity in the isolated rats was reinstated. Determination of brain tissue concentration of monoamines (DA, NE, and 5-HT) and their metabolites from similarly reared, isolated (individual cages) and grouped rats (6 per cage), indicated a significant reduction in frontal cortex dopamine activity in isolates. This is in confirmation of earlier studies on the effects of isolation housing (Blanc et al, *Nature* 284 1980). This experiment therefore also examined the effects of frontal cortex dopamine depletion (induced by 6-OHDA) on locomotor activity conditioned to the presentation of food, as a comparison with the isolation-induced reduction in mesocortical DA activity. These results will be discussed in terms of the effects of social-isolation on central dopamine function and behaviour.

390.15

EFFECT OF ORAL ASPARTAME ON RAT EXPLORATORY BEHAVIOR FOLLOWING STRESS. H.H. Wichman*, N.H. Copp*, and J.B. Eck* (SPON: A. Jones). Claremont McKenna College, Claremont, CA 91711

It has been shown that combined tail-shock and immobilization depletes norepinephrine in the rat hypothalamus, and depresses locomotor and exploratory behavior. Oral tyrosine ingestion blocks this behavioral depression. Since oral Aspartame also produces increased blood levels of tyrosine it ought to have an effect similar to tyrosine alone. This study had two purposes: A) To determine whether immobilization stress done without shock produces post-stress behaviorial depression; and B) to determine whether Aspartame ingestion would block post-stress behavioral depression of exploratory behavior. Forty Sprague-Dawley rats were fed a high carbohydrate meal dosed with Aspartame (200 mg/kg). Two hours of immobilization stress followed. Immobilization stress depressed all behavior--validating the more humane (non-electric shock) stress stimulus. In the Aspartame condition the post-stress depression was significantly blocked, thus demonstrating a post-stress behavioral effect of Aspartame ingestion not previously reported.

390.16

STRAIN-SPECIFIC CATECHOLAMINE VARIATIONS INDUCED BY STRESSORS: RELATION TO BEHAVIORAL CHANGE. N. Shanks, S. Zalcman*, C.R. Prince*, and H. Anisman. Dept. of Psychology, Carleton University, Ottawa, Ont. Canada K1S 5B6.

Exposure to inescapable shock provoked region-specific alterations of norepinephrine (NE) and dopamine (DA) activity and levels which varied across strains of mice. In the mesolimbic frontal cortex and nucleus accumbens shock induced an increase of DOPAC accumulation and pronounced reductions of DA in some strains, while in others these variations were less pronounced or entirely absent. Likewise, the stressor provoked reductions of NE and increases of MHPG accumulation in hypothalamus, hippocampus and locus coeruleus varied appreciably across strains. Strain-specific disturbances of shuttle escape performance associated with inescapable shock corresponded relatively well with the MHPG accumulation in the locus coeruleus and the DA reductions in the frontal cortex. Likewise, stressor-provoked alterations of self-stimulation from the nucleus accumbens were related to DOPAC accumulation in this region.

390.17

ALTERATIONS IN CENTRAL DOPAMINE PATHWAYS INDUCED BY SELECTIVE BREEDING FOR AGGRESSION AND BY SOCIAL EXPERIENCE. M.H. Lewis, J.L. Gariepy*, S. Southerland*, R.B. Mailman, and R.B. Cairns*. Biol. Sci. Res. Ctr. and Depts. of Psychiatry, Pharmacology, and Psychology, Univ. of North Carolina, Chapel Hill, NC 27514

A selective breeding strategy has resulted in rapid, persistent line differences in mice from a common genetic background. NC900 mice exhibit high levels of species-typical, isolation-induced aggression when evaluated in a social interaction test. Conversely, NC100 mice exhibit little aggression, but high levels of immobility. In both lines, even one exposure to the social interaction test decreases the latency to attack. We hypothesized that central dopamine pathways, altered by selective breeding, mediated, at least in part, this line difference. Mice from both lines, half of which had been exposed to the social interaction test, were sacrificed four days after testing. Concentrations of dopamine and its acidic metabolites, DOPAC and HVA were quantified using microdissected samples from various brain regions. Significant effects for both line and social experience were found but with no interaction. NC100 mice had significantly decreased concentrations of DOPAC and HVA, in both the caudate nucleus and amygdala. Irrespective of line, exposure to the social interaction test resulted in substantial increases in dopamine utilization in the caudate and amygdala. The present results support the hypothesis that central dopamine pathways mediate line differences, and also lead to the hypothesis that social experience results in persistent alterations in dopamine utilization, perhaps analogous to that described for amphetamine sensitization. (Supported by PHS Grants HD14648 and HD03110.)

390.18

FETAL TRANSPLANTS AS A TOOL FOR STUDYING FUNCTIONAL HETEROGENEITY OF THE DOPAMINE SYSTEM. J.B. Richards and C.R. Freed, Depts. of Med. and Pharm., Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

Functional heterogeneity of the dopamine (DA) system has typically been studied using localized 6-hydroxydopamine (6-OHDA) lesions and intracranial injections of DA agonists and antagonists. The transplantation of fetal DA cells provides another technique for studying functional heterogeneity of the DA system. In this study, rats were unilaterally lesioned with 6-OHDA and then tested for amphetamine (AMPH) induced turning with 5 mg/kg methamphetamine. Rats which did not meet a criterion of >5 turns per min were not used. The rats received transplants in the nucleus accumbens (NAS), dorsal striatum (DS), and ventral striatum (VS). The effects of DA transplants on AMPH induced turning, locomotion, and stereotypy were observed. Preliminary data showed that NAS transplants caused an increase in AMPH induced locomotion. Transplants in the DS reversed direction of AMPH induced turning. Transplants in the VS decreased the rate of AMPH induced locomotion and turning and increased the amount of time spent in stationary stereotypy.

390.19

SLEEP DEPRIVATION (SD) AND YOHIMBINE INCREASE THYROTROPIN (TSH) SECRETION IN HUMANS. N.Sucher* and A.Baumgartner*(SPON:ENA). Psychiatrische Klinik, Freie Universität Berlin (West), Germany.

A whole night's sleep deprivation improves mood in depressed patients. Nothing is known about the mechanisms involved. Since thyroid disorders have also often profound effects on mood, we investigated changes in TSH during and after sleep deprivation in depressed patients and healthy males.

In 50 patients with major depression, TSH concentration was 1.4+1.35mU/l at 8 a.m. before and 2.14+ 1.84mU/l after SD. The rise in TSH levels was significantly correlated to response measured as changes in scores of visual analog mood scales.

TSH secretion is believed to be under central noradrenergic control. We, therefore, investigated the effects of oral prazosine, yohimbine, and propanolol (a_1, a_2, and ß adrenoceptor antagonists) on TSH secretion in 6 healthy males during SD. TSH concentration rose during SD. Prazosine and propanolol had no effect. Yohimbine led to a significant further increase in TSH secretion, probably induced by antagonistic action on presynaptic a_2 receptors, thereby leading to enhanced norepinephrine activity. We conclude that at least the observed neuroendocrine effect of SD is mediated by increased noradrenergic activity, probably due to activation of noradrenergic neurons in the locus coeruleus.

390.20

BEHAVIORAL EFFECTS OF ACUTE TRYPTOPHAN DEPLETION IN DEPRESSED AND OBSESSIVE CUMPULSIVE DISORDER (OCD) PATIENTS. P.L. Delgado, D.S. Charney, L.H. Price, W.K. Goodman, G.K. Aghajanian, H. Landis*, G.R. Heninger, Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508

Reduction of dietary tryptophan (TRP) decreases plasma TRP, brain TRP, and brain serotonin (5HT) in laboratory animals. In healthy humans, it produces mild impairment of attentive performance, reports of negative mood, enhancement of the prolactin response to I.V. TRP, and reduction of CSF 5-HIAA. METHOD: 32 patients (13 depressed and 7 OCD, drug free; 12 previously depressed, now remitted on antidepressant) received two tests, each consisting of a 24-hr., 160 mg/day, low-TRP diet followed the next morning by administration of a 16-amino acid drink, in a double-blind, placebo-controlled (acute or sham TRP depletion), crossover fashion. On one test the diet was supplemented with 500 mg L-TRP tid and the drink contained 2.3 gm L-TRP (sham depletion) and on the other test the diet and drink were not supplemented with TRP (acute TRP depletion) Four of the 25 depressed patients (three remitted and 1 drug-free) were tested single-blind with no sham test. Behavioral ratings and plasma (for free and total TRP levels) were obtained prior to starting the diet and prior to and 5 to 7 hrs. after the drink. Normal TRP intake resumed 7 hrs. after the drink in both groups of patients. Patients were again rated at 12:00 PM the day following the drink. RESULTS: Total and free TRP were decreased 90% 5 hrs. after the TRP-free drink (p<.0001). Nine of 13 drug-free depressed patients improved (50% Hamilton Depression Scale (HDRS) decrease), and 4 worsened (22% HDRS increase) the day following acute, but not sham TRP depletion. Eight of 12 remitted depressed patients on antidepressant relapsed (374% HDRS increase; HDRS: 9±4 pre-diet, 29±6 after drink) following acute, but not sham TRP depletion. OCD patients were behaviorally unchanged by acute or sham TRP depletion. Implications: Acute TRP depletion followed by resumption of normal TRP intake leads to rapid improvement of depressive symptoms in most drug-free depressed patients. Relapse of remitted depressed patients suggests that the therapeutic effects of some antidepressant drugs (desipramine, fluvoxamine, and tranylcypromine) may depend on 5HT availability. OCD may be pathophysiologically distinct from depression. Acute TRP depletion is a useful method with which to evaluate 5HT function in humans.

CARDIOVASCULAR REGULATION VI

391.1

PREOPTIC PERIVENTRICULAR LESIONS DECREASE VASCULAR CAPACITANCE IN THE RAT. S.L. Bealer, Dept. Physiology, Univ. Tennessee, Memphis, TN 38163

Ablation of tissue surrounding the anteroventral portion of the third cerebral ventricle (AV3V) produces decreased plasma volume, decreased vasoconstrictor reserve, and acute changes in hemodynamics characteristic of increased central blood volume in the rat. The present experiments were designed to determine if AV3V ablation results in decreased vascular capacitance, which could account for these cardiovascular responses. Following recovery from either electrolytic ablation of the AV3V region (AV3V-X) or control surgical procedures (CONT), mean circulatory filling pressure (MCFP) was determined in anesthetized animals during circulatory arrest. MCFP was significantly greater in AV3V-X rats (7.8 ± 0.3 mmHg) than in CONT animals (5.2 ± 0.3 mmHg). Furthermore, intravenous infusion of whole blood (50% estimated blood volume over 15 min) resulted in a significantly greater pressor response (16 ± 4 mmHg) and natriuresis (154 ± 25 µEq) in AV3V-X rats than in CONT animals (4 ± 4 mmHg; 60 ± 10 µEq) during the 30 min following infusion. These data are consistent with the hypothesis that AV3V ablation results in decreased vascular capacitance. (Supported by USPHS grants HL-25877, HL-01237, and from the American Heart Association)

391.2

HYPOTHALAMIC INJECTIONS OF OUABAIN ELICIT PRESSOR RESPONSES IN ANAESTHETIZED RATS. D.L. Jones. Depts. of Physiol. & Med., Univ.of Western Ontario, LONDON, Ontario, Canada, N6A 5C1.

Cardiac glycosides are known to have a narrow therapeutic index, due in part to their effects on the brain. Injections into the ventricles of the brain elicit activation of the autonomic nervous system. However, the specific regions in the brain responsible for such action are unknown. The purpose of this experiment was to map the rostral diencephalon to determine sites at which injections of a low dose of the cardiac glycoside, ouabain, resulted in altered cardiovascular responses in the anaesthetized rat. Microinjections of 20 ηg of ouabain in 250 ηL were made into various rostral diencephalic sites of urethane (1.2g/kg) anaesthetized rats while monitoring heart rate and blood pressure. Injections into the nucleus medianus, periventricular, paraventricular, dorsalmedial, lateral and posterior hypothalamic nuclei produced increases in pressure of from 5 to 25 mm Hg, while similar injections outside these regions were without effect. These data suggest that part of the toxicity resulting from the cardiac glycoside administration may be due to the direct action of the glycosides on these hypothalamic structures.

Supported by the Heart and Stroke Foundation of Ontario.

391.3

INCREASE IN BLOOD PRESSURE AND RENAL SYMPATHETIC OUTFLOW BY STIMULATION OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS IN CONSCIOUS RATS. H. Kannan[1], Y. Hayashida*[2], X. J. Jin*[1] and H. Yamashita[1] Dept. of Physiol.[1], and Dept. of Systems Physiol.[2], Univ. of Occup. Environ. Health, Sch. of Med., Kitakyushu, Japan 807.

The paraventricular nucleus (PVN) of the hypothalamus has been suggested to participate in control of autonomic nerve activity. Our previous studies(J. Auto. Nerv. Syst., 19:229-234, 1987 and 21:83-86, 1987) demonstrated in anesthetized rats that PVN stimulation resulted in depressor responses accompanied with decreases in sympathetic outflow. Because anesthesia may alter sympathetic responses and cardiovascular reflexs, we decided to examine the effects of PVN stimulation on blood pressure and renal sympathetic nerve activity in conscious rats. Electrical stimulation through chronically implanted electrode evoked increases in blood pressure and renal sympathetic nerve activity with a slight decrease in heart rate. These responses were dependent upon the frequency and the intensity of the stimulus. Latency of the excitatory response of the renal sympathetic nerve activity was approximately 70 ms. Microinjection of L-glutamate (0.5M, 200nl) into the PVN also elicited increases in blood pressure and renal sympathetic nerve activity. The result suggests that activation of PVN neurons in conscious rats leads to pressor responses due to increasing sympathetic outflow, which contrast with those obtained previously in anesthetized rats.

391.4

MICROINJECTION (MI) OF NEUROPEPTIDE Y (NPY) INTO THE POSTERIOR HYPOTHALAMIC NUCLEUS (PHN) INCREASES ARTERIAL PRESSURE (AP) AND RENAL SYMPATHETIC NERVE ACTIVITY (RSNA). J.R. Martin, M.M. Knuepfer and T.C. Westfall. Dept. of Pharmacol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

We have reported that MI of NPY into the PHN of urethane-anesthetized rats results in an increase in AP. The present study was undertaken to characterize the mechanism by which this increase occurs. Urethane-anesthetized rats were prepared for AP measurement and intravenous drug administration. A cannula was placed in the PHN for MI of carbachol (CARB, 5.5 nmol) or NPY (2.4 nmol), and RSNA was recorded by rectifying and integrating multiple unit activity of a renal nerve. MI of CARB or NPY into the PHN increased AP and RSNA (see table). The increase in AP could be blocked by pretreatment with the ganglionic blocker pentolinium (PENT, 7.5 mg/kg, i.v.) but not by a V_1 vasopressin receptor antagonist (V_1 ANT, 10 µg/kg, i.v.).

Peak Changes in AP (mmHg) or RSNA (%)

	AP Experiments (N)			RSNA Experiments		
	CONTROL	+ PENT	+V_1 ANT	AP	N	RSNA
NPY	12 ± 3 (14)	1 ± 1 (6)	21 ± 3 (6)	12 ± 3	7	30 ± 9
CARB	21 ± 1 (15)	4 ± 1 (6)	30 ± 3 (6)	20 ± 4	6	55 ± 16

Saline MI had no effect on AP or RSNA. We conclude that the increase in AP produced by MI is likely due to sympathoexcitation. (Supported by HL26319, HL35202, NS07254, HL38299 and an AHA MO Affiliate fellowship.)

391.5

EFFECTS OF SINOAORTIC NERVE DENERVATION ON THE RELEASE OF ENDOGENOUS NOREPINEPHRINE FROM THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS OF UNANESTHETIZED FREELY MOVING RATS. J.M. Qualy* and T.C. Westfall (SPON: S. Horenstein). Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

We previously reported enhanced basal and K^+ stimulated release of norepinephrine (NE) from the PVH in 7-10 wk old SHR as compared to age matched WKY and SD rats. This increased release was attenuated in 12 wk old SHR suggesting enhanced noradrenergic neuronal activity during the developing phase of hypertension. In addition, a reciprocal relationship exists with respect to blood pressure and NE release such that an increase/decrease in blood pressure resulted in a decrease/increase of PVH NE release. These results suggested that the noradrenergic pathways of the PVH contribute to the maintenance of arterial blood pressure homeostasis presumably via the baroreceptor reflex. The present study examined the effects of sinoaortic denervation (SAD) on PVH NE release. The reciprocal relationship between blood pressure and NE release was abolished after SAD. The administration of phenylephrine no longer attenuated the release of NE, and the administration of sodium nitroprusside no longer exacerbated PVH NE release. These results demonstrate that an intact baroreflex is necessary for the noradrenergic pathways of the PVH to contribute to the maintenance of arterial blood pressure homeostasis. (Supported by NIH grants HL35202 and HL26319.)

391.6

PRESSOR AND VASOCONSTRICTOR RESPONSES PRODUCED BY L-GLUTAMATE IN THE LATERAL HYPOTHALAMUS OF CONSCIOUS RATS. A.-L. Sirén, R. Press* and G. Feuerstein. Dept. of Neurol., USUHS, Bethesda, MD 20814.

The effects of microinjections (50 nl) of l-glutamate (GLU) into lateral hypothalamus (LH) were studied in conscious male Sprague-Dawley rats (n=7, 340-360g). Mean arterial pressure (MAP), heart rate, blood flow (BF) and vascular resistance (VR) in hindquarter (HQ), renal (R) and mesenteric (M) vessels were monitored. The injection site and spread were confirmed on thionin stained sections (50μ) and by a radioactive tracer. GLU (100 nmol) in LH produced a sustained pressor response (+33±7 mmHg, $p<0.01$). The increase in MAP became apparent in 1-2 min and subsided in 10-15 min. In 4/7 animals the pressor response was accompanied by an initial decrease in heart rate (-52±18 bpm, $p<0.05$), and in all animals by a sustained tachycardia (+81±15 bpm, $p<0.01$). The HQBF first decreased (-22±2%, $p<0.05$) and then increased (+37±9%, $p<0.01$) while HQVR increased (+51±9%, $p<0.01$). The BF decreased in R and M blood vessels by -28±7% ($p<0.01$) and -30±12% ($p<0.05$), due to an increase in RVR (+73±34%, $p<0.05$) and MVR (+68±21%, $p<0.05$). The results demonstrate that in the conscious rat GLU in LH produces pressor and vasoconstrictor responses, not depressor responses as reported in the anesthetized rat. It is suggested that GLU receptors in LH mediate the pressor response evoked by electrical stimulation of the LH area.

391.7

LATERAL HYPOTHALAMIC AREA: ROLE IN CARDIOVASCULAR CONTROL S.E. Spencer, W.B. Sawyer*and A.D. Loewy, Depts. of Neurology and Anatomy & Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

We have previously reported that L-glutamate stimulation of the lateral hypothalamic area (LHA) in rats lowers both blood pressure and heart rate (Soc. Neurosci. Abst., 13(1): 282, 1987). Stimulation of the subthalamic nucleus and dorsal zona incerta did not alter blood pressure or heart rate; while ventral zone incerta stimulation generated hypotension and bradycardia. We reported that this LHA influence on blood pressure is mediated by reductions in cardiac output and is not a result of decrease in total peripheral resistance (FASEB J., 2(6): A1728, 1988).

In our initial studies, we observed a rostrocaudal organization within the LHA. This report extends these studies to discern potential differences after stimulating different LHA subdivisions or the perifornical hypothalamic area.

Stimulating the tuberal region of the LHA (LHA_t), which lies lateral to the ventromedial nucleus of the hypothalamus, decreased the heart rate and blood pressure. In contrast, stimulating the posterior part of the LHA (LHA_p), which lies caudal to the LHA_t and lateral to the mamillary nuclei, decreased the heart rate and blood pressure minimally. Stimulation of the perifornical hypothalamic area caused a fall in blood pressure but no change in heart rate.

The use of pharmacological blockers permitted a determination of whether the LHA_t bradycardia was sympathetically or vagally mediated. Timolol eliminated the bradycardic response. Atropine reduced the bradycardic response by 35%.

Our results show that the LHA_t influences both heart rate and blood pressure while the LHA_p influences neither. The adjacent perifornical area affects blood pressure but not heart rate.

391.8

EFFECT OF SYMPATHETIC NERVOUS SYSTEM ON DIURNAL VARIATION OF CARDIOVASCULAR PARAMETERS IN CONSCIOUS MONKEYS. M.I. Talan and B.T. Engel*. Lab. of Behavioral Sciences, Gerontology Research Center, NIA, Baltimore, MD 21224.

Heart rate, stroke volume, and intra-arterial blood pressure were monitored continuously in each of four monkeys, 18 consecutive hours/day for several weeks. Mean heart rate, stroke volume, cardiac output, systolic and diastolic blood pressure, and total peripheral resistance were calculated for each minute, and reduced to hourly means. After baseline data were collected for approximately 20 days, observation was continued for equal periods of times under conditions of α-sympathetic blockade, β-sympathetic blockade, and double sympathetic blockade achieved by intra-arterial infusion of atenolol, prazosin, or a combination of both in concentration sufficient for at least 75% reduction of response to injection of respective agonists. A stable diurnal pattern of hemodynamic function was observed in the control condition: cardiac output fell throughout the night, primarily as a result of a fall in heart rate since stroke volume was relatively unchanged; a small reduction in blood pressure also occurred; however, this fall was buffered by a rise in peripheral resistance which paralleled the fall in cardiac output. Average overnight total peripheral resistance was 49% greater during atenolol infusion and 20% greater during double blockade. The hemodynamic pattern was not eliminated by selective blockade of α- or β-sympathetic receptors, or by double sympathetic blockade; in fact, it was exacerbated by sympathetic blockade indicating that the sympathetic nervous system attenuates these events. The fact that sympathetic blockade did not eliminate this pattern suggests that a diurnal loss in plasma volume may mediate the fall in cardiac output, and that the rise in total peripheral resistance reflects autoregulation of arterial pressure.

391.9

CHANGES IN ARTERIAL BLOOD PRESSURE AND RENAL BLOOD FLOW DURING SERIAL SEIZURES IN THE RAT. R.M. Zweifler*, E.M. Slaven*, J.C. Magee*, and N.R. Kreisman. Dept. of Physiol., Tulane Univ. Sch. Med., New Orleans, La 70112.

In status epilepticus, mean arterial blood pressure (MABP) increases during early seizures but decreases during later seizures (Glaser Adv. in Neurol. 34: 395-398, 1983; Kreisman et al., Adv. in Neurol. 34: 231-239, 1983). Renal blood flow (RBF) was measured during serially induced seizures in anesthetized, paralyzed rats to determine whether the fall in ictal MABP results from loss of tone in the renal vascular bed. During early seizures, ictal MABP increased nearly 50% while (RBF) transiently decreased nearly 100%. With subsequent seizures, the rises in MABP either diminished or remained the same while the decreases in RBF became smaller. RBF became pressure passive when later seizures were accompanied by a decrease in MABP. In contrast, i.v. administration of norepinephrine during interictal periods always produced profound renal vasoconstriction, indicating that the renal vascular bed remained responsive. The importance of these changes in the renal vascular response to serial seizures will be discussed. (Supported by NIH NS-17443)

391.10

CENTRAL CARDIOVASCULAR ACTIONS OF CORTICOTROPIN RELEASING FACTOR IN SINOAORTIC DEAFFERENTED RATS. J.M. Overton* and L.A. Fisher. Dept. of Pharmacology, Coll. Med., University of Arizona Health Sciences Center, Tucson, AZ 85724.

Corticotropin releasing factor (CRF) acts within the central nervous system (CNS) to elevate arterial pressure (AP) and heart rate (HR) simultaneously and markedly inhibit reflex bradycardia in response to increased AP. If these cardiovascular changes are mediated by inhibition of impulse transmission of baroreceptor afferent fibers terminating within the CNS, prior removal of such afferents should preclude the ability of CRF to alter cardiovascular function. To examine this possibility, the CNS cardiovascular actions of CRF were measured in rats after sinoaortic deafferentation (SAD). All experiments utilized conscious, unrestrained male rats previously instrumented with lateral cerebroventricular (icv) cannulae for peptide administration and femoral arterial catheters for direct measurement of AP and HR. Sham (n=6) and SAD (n=11) operations were performed in a one-stage procedure two weeks prior to experiments. SAD abolished phenylephrine-induced reflex bradycardia (for a 20 mm Hg rise in AP, HR fell by 3±1 vs. 41±5 bpm in shams). Resting AP (114±3 vs. 101±2 mm Hg) and HR (403±8 vs. 363±5 bpm) were slightly elevated after SAD vs. sham operation. Maximal elevations of AP and HR after CRF (0.15 nmole, icv) administration were 15±2 mm Hg (15%) and 93±15 bpm (25%) in sham-operated rats vs. 23±4 mm Hg (20%) and 64±7 bpm (16%) in SAD-operated rats. These results suggest that although CRF-induced elevations of HR may be attenuated after SAD, this peptide acts in the CNS to increase AP and HR via a mechanism apart from inhibition of baroreceptor afferent transmission.

391.11

RECOVERY OF BAROREFLEX CONTROL OF HEART RATE AFTER RIGHT UNILATERAL VAGOTOMY IN RABBITS. D.D. Lund, B.J. Pardini, A.R. Subieta, G.A. Davey, and P.G. Schmid. V.A. Medical Center, Cardiovascular Center and Dept. of Internal Med., University of Iowa, Iowa City, IA 52240.

The time-course of recovery of arterial baroreflex control of heart rate after unilateral mid-cervical vagotomy was evaluated by measuring the baroreflex sensitivity in urethane-anesthetized, propranolol-treated rabbits. Baroreflex sensitivity was measured as the slope of the regression line between the R-R interval (msec) and mean arterial pressure (mm Hg) during a 30 sec linear ramp change in arterial pressure. The ramp was generated by a an electronic negative feedback device that controlled the infusion rate of phenylephrine from control pressure to 150 mm Hg. There was a significant reduction ($p<0.05$) in the RR/BP response to phenylephrine after an acute right midcervical vagotomy (acute vagotomy = 0.97±0.14 msec/mm Hg vs. Control = 1.82±0.33 msec/mm Hg). This response was still significantly reduced from control at 1 week following vagotomy (1.21±0.24 msec/mm Hg). At 4 weeks following vagotomy the baroreflex sensitivity had returned to control levels (3.4±1.22 msec/Hg). No changes were found in the response to methyl choline indicating that alterations in the muscarinic cholinergic receptors could not account for recovery of baroreceptor reflex response after vagotomy. These data support the concept that recovery of baroreceptor reflex response may be related to compensatory changes in the intact contralateral vagus nerve and not an alteration of cholinergic muscarinic receptors. Supported by Vet Administration and NIH HL38137.

391.12

RECOVERY OF HEMODYNAMIC FUNCTION IN CONSCIOUS RATS AFTER SYSTEMIC 6-HYDROXYDOPAMINE (6HDA). J.M. Sved, S.P.Corey*, E.M. Stricker and R.R. Vollmer*. Depts. Behavioral Neuroscience and Pharmacology, Univ. Pittsburgh, Pittsburgh, PA 15260

These experiments investigated the recovery of neural control of blood pressure (BP) and heart rate (HR) in rats one week after administration of 6HDA (100 mg/kg, sc). Catheters were placed in the femoral artery and vein at least 24 hr before experiments. BP was reduced in the 6HDA-treated rats (104±4 mmHg vs 88±3 mmHg, $p<0.01$) whereas HR was not affected. In plasma samples obtained while the rats rested quietly, norepinephrine (NE) levels were significantly less in the 6HDA-treated rats (211±32 pg/ml vs 128±15 pg/ml, $p<0.05$) whereas epinephrine levels were similar in both groups. In response to an infusion of sodium nitroprusside (2.5 ug/kg/min, iv), BP decreased to 62±2 mmHg in vehicle-treated rats and to 44±3 mmHg in 6HDA-treated rats; yet a similar tachycardia was produced in both groups. At the conclusion of the experiments, ventricular and atrial NE content in the 6HDA-treated rats was found to be 91% and 86% of control values, respectively. These results in the conscious rat agree with our observations in pithed rats (Fluharty et al., J Pharm Exp Ther, 243:415, 1987), in which BP and NE increments to electrical stimulation were found to be significantly impaired whereas increases in HR were normal. (Supported by HL26212 and NS19608.)

391.13

CANINE MYOCARDIAL CELL SURFACE ß-ADRENERGIC RECEPTORS ARE NOT ALTERED DURING PROLONGED MAXIMAL STELLATE GANGLIONIC STIMULATION

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Electrical stimulation of thoracic sympathetic ganglia results in increased inotropism which rapidly peaks and then becomes diminished within a few minutes, even though stimulation continues. This inhibition could be due to a decreased availability of myocardial ß-adrenergic receptors. A myocardial slice preparation (Haddad et al., Can. J. Physiol. Pharmacol. 67:1928, 1987) was utilized to assess the binding characteristics of the hydrophilic ß-adrenergic antagonist, [^{3}H] CGP-12177, to cell surface receptors in tissues which had been removed from the ventral midwall of the left ventricle (LVV) and the right ventricular conus (RVC) prior to maximal stimulation (15V, 10 Hz, 4 ms) of acutely decentralized stellate ganglia of mongrel dogs (PRE) as well as after 20 minutes of stimulation while stimulation continued (20 MIN). Stimulation increased intramyocardial pressure (IMP) in LVV from 78±7 to 258±23 mm Hg initially but after 20 minutes of continuous stimulation it was 90±16 mm Hg. IMP in RVC followed a similar pattern. Bmax in LVV prior to stimulation was 14.61±0.94 fmol/mg wet wt and was not altered after 20 minutes stimulation (14.09±0.62 fmol/mg wet wt)($p>0.05$). Kd likewise was similar PRE (0.29±0.05 nM) versus 20 MIN (0.28±0.05 nM). In RVC, both Bmax (15.21±1.89 fmol/mg wet wt) and Kd (0.32±0.09 nM) were similar to those in LVV prior to stimulation ($p>0.05$) and neither was altered as a result of stimulation. These results demonstrate that 20 minutes of maximal electrical stimulation of canine stellate ganglia does not result in a loss of ß-adrenergic receptors from the myocardial cell surface, and suggest that an acute increase in sympathetic function does not lead to alteration of myocardial ß-adrenergic receptors.

Supported by the Nova Scotia Heart Foundation and MRC of Canada

391.14

DEVELOPMENT OF A1 ADENOSINE RECEPTORS AND RESPONSIVENESS OF ADENYLATE CYCLASE TO INHIBITION BY CYCLOPENTYLADENOSINE IN EMBRYONIC CHICK HEART. T.A.Blair* and T.F.Murray. Oregon State Univ., Corvallis, OR 97330

The developing chick heart has been used to study the biochemical events involved in the activation and regulation of various neuroreceptors. In the present study we have used the embryonic chick heart to study the development of A1-adenosine receptors and their interaction with adenylate cyclase during embryogenesis. Temporal correlation of these results with previous physiological studies permits inferences to be made concerning the relationship between the development of sensitivity to adenosine analog-induced negative chronotropic response and the appearance of A1 receptors coupled to adenylate cyclase. At 5- and 7-days in ovo the number of receptors in cardiac membranes, as determined by the binding of [3H]cyclopentyldipropylxanthine, appear to have similar densities. Between day-7 and 9 the density increases 1.5 to 2 fold and remains relatively constant through day-16. Equilibrium saturation analysis demonstrated that the Bmax values were 46.8±1.3fmol/mg protein for 7 days, 74.75±6.5 fmol/mg protein for 9 days and 69.2 ±1.6 fmol/mg protein for 14 days in ovo with no apparent change in receptor affinity. Despite these developmental increases in the densities of adenosine receptors there were no corresponding changes in A1-receptor mediated inhibition of basal adenylate cyclase activity between embryonic days 4 and 16. The maximum inhibition obtained using the A1-selective agonist cyclopentyladenosine ranged from 9.2 ±.4% to 14.2±1.2% with IC50 values of 0.22 ± 0.11 to 2.8±0.92 uM. From these data we conclude that the increase in A1 adenosine receptors as a function of ontogeny may partially explain the increase in physiological response seen during embryogenesis. The sensitivity to A1-receptor mediated inhibition of adenylate cyclase is not temporally correlated with physiological responsiveness and therefore does not appear to be involved in the A1-receptor mediated negative chronotropic response.

391.15

AGONIST RADIOLIGAND INTERACTIONS WITH THE SOLUBILIZED PORCINE ATRIAL ADENOSINE RECEPTOR. M. Leid and T.F. Murray, Coll. of Pharmacy, Oregon State Univ., Corvallis, OR 97331.

The agonist radioligand [^{125}I]hydroxyphenylisopropyladenosine ([^{125}I]HPIA) was used to characterize solubilized cardiac adenosine receptors (cAdoR). Porcine atrial membranes were solubilized using a mixed detergent system consisting of 0.4% w/v digitonin and 0.08% w/v cholate. [^{125}I]-HPIA showed saturable binding to an apparently homogenous population of solubilized recognition sites with a Bmax of 25 ± 1 fmol/mg protein and a K_D of (6.9 ± 0.8) x 10^{-10} M. The results of kinetic experiments suggest that [^{125}I]HPIA interacts with solubilized cAdoR in a simple bimolecular reaction. Guanyl nucleotides negatively modulated the binding of [^{125}I]HPIA indicating that solubilized cAdoR maintain the ability to interact with guanyl nucleotide binding protein(s). Competition binding experiments using adenosine receptor agonists and antagonists as inhibitors of [^{125}I]HPIA binding suggest that the pharmacological specificity of solubilized cAdoR has been preserved. In summary, [^{125}I]HPIA bound saturably and reversibly to the solubilized cAdoR via an apparent simple bimolecular reaction. Both the pharmacological specificity of the cAdoR and its ability to interact with guanyl nucleotide binding protein(s) are maintained in this detergent system. Supported by a grant from the Oregon Affiliate of the American Heart Association. ML is a H.A.B. Dunning Memorial Fellow of the American Foundation for Pharmaceutical Education.

391.16

RELATIONSHIP OF SYMPATHETIC NERVE FIBERS AND MAST CELLS OF THE RAT DURA MATER. **KELLER, J.T., R.Y.W. DIMLICH, M. ZUCCARELLO*, B.E. TIERNEY*, Depts. of Neurosurg.,Emerg. Med., and Anat./Cell Biol., U. of Cincinnati, and J.N. Gamble Inst. of Med. Res., and The Christ Hospital, Cincinnati, Ohio.**

The neural elements associated with meningeal vasculature are similar to those of the cerebral vessels at the base of the brain. We are studying the relationship of nerve fibers associated with dural vasculature as a model to gain an understanding of the mechanisms which govern cerebral blood flow and cephalgias. Because a prominent perivascular mast cell population is associated with the meninges, consideration is given to the relationship of these cells to the sympathetic nervous system. Previous ultrastructural investigations have demonstrated an association between mast cells and nerve fibers, including those of the sympathetic nervous system, in other tissues. The purpose of this study was to: 1. examine the sympathetic innervation of the rat meninges in normal animals and those in which the superior cervical ganglion (SCG) had been bilaterally extirpated, and, 2. examine the mast cell population in these two groups of animals. Using the glyoxylic acid (GA) technique of de la Torre, whole mount preparations of supratentorial dura mater were examined for the presence of catecholamine fibers and mast cells. Robust plexuses of noradrenergic fibers and perivascular mast cells were associated with the middle meningeal artery and its branches. In five animals, examined seven days following bilateral extirpation of the SCG, noradrenergic nerve fibers were no longer identified and the mast cell population had decreased in number as visualized using the GA fluorescent technique. These preliminary results are suggestive that the sympathetic nervous system may have a modulatory influence on meningeal mast cells. These observations warrant further investigation.

392.1

CENTRAL α_2 ADRENERGIC MECHANISMS IN THE RENAL NERVE MEDIATED NATRIURESIS AND DIURESIS PRODUCED BY ACUTE VOLUME EXPANSION. KP Patel* (SPON: SB Waller) Dept. of Physiol. & Pharm., University of South Dakota, Vermillion, SD 57069.

To determine whether central α_2 adrenergic mechanisms are involved in the natriuresis and diuresis produced by acute volume expansion (VE), urine flow ($\dot{v}$) and sodium excretion ($UNa\dot{v}$) from innervated and denervated kidneys were measured before and after VE (1 ml/min for 20 min) in presence or absence of intracerebroventricular yohimbine (8 µg/kg/min) in Inactin® anesthetized Sprague Dawley rats. The innervated to denervated (I/D) ratio for $\dot{v}$ and $UNa\dot{v}$ indicated that VE caused a greater natriuresis and diuresis from the intact compared to the denervated kidney.

		Control	Volume Expansion
No Yohimbine	$\dot{v}$	0.23 ± 0.03	0.61 ± 0.10
	$UNa\dot{v}$	0.21 ± 0.03	0.61 ± 0.09
Yohimbine	$\dot{v}$	0.24 ± 0.04	0.36 ± 0.07*
	$Una\dot{v}$	0.21 ± 0.04	0.34 ± 0.07*

Data represent mean I/D ± SEM, *$p<0.05$, No yohimbine vs. yohimbine (n=7). Central administration of yohimbine blunted the increase in the I/D ratio to VE. These data suggest that central α_2 adrenergic mechanisms may be part of the renal sympatho-inhibition responsible for natriuresis and diuresis produced by acute VE.

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392.2

THE INVOLVEMENT OF α_1- AND α_2-ADRENOCEPTORS OF NUCLEUS RETICULARIS GIGANTOCELLULARIS IN THE ANTIHYPERTENSIVE EFFECTS OF GUANABENZ. H.C. Lim* and S.H.H. Chan.(SPON: S.F. LEONG) National Univ. of Singapore, Kent Ridge, Singapore 0511, and National Yang-Ming Medical Coll., Taipei 11221, Taiwan.

Guanabenz, like clonidine, is thought to exert its antihypertensive effects via the central α-adrenoceptors. Since the nucleus reticularis gigantocellularis (NRGC) has been shown to be critically engaged in clonidine- and guanabenz-induced hypotension, we investigated the involvement of α-adrenoceptors in the NRGC in the cardiovascular effects of guanabenz, using pentobarbital anesthetized male Sprague-Dawley rats. Pretreatment with α-adrenoceptor antagonists yohimbine (10 µg), phentolamine (2.5 µg) and phenoxybenzamine (20 µg), which were microinjected into bilateral NRGC, significantly antagonized the cardiovascular suppressive effects normally elicited by an intravenous administration of guanabenz (10 µg/kg). In contrast, pretreatment with prazosin (0.25 µg) did not affect the hypotensive, but inhibited the bradycardiac actions of guanabenz. Pretreatment with either clonidine (0.5 µg) or guanabenz (0.5 µg) significantly potentiated the initial hypertension, and antagonized the vasodepression, normally promoted by the other compound. We conclude that guanabenz may produce antihypertension by activating the α_2-adrenoceptors, and to a lesser extent, the α_1-adrenoceptors, in the NRGC.

392.3

INTRATHECAL CLONIDINE PRODUCES PRESSOR EFFECTS BY PERIPHERAL REDISTRIBUTION AND ACTIVATION OF VASCULAR ALPHA-ADRENOCEPTORS. G.F. Gebhart, R.E. Solomon and R.K. Bhatnagar, Department of Pharmacology, University of Iowa College of Medicine, Iowa City, IA 52242.

Intrathecal (i.t.) clonidine in rats produces, at lesser doses (1.0 - 10.0 µg), depressor and antinociceptive effects mediated by spinal α_2-adrenoceptors and, at a greater dose (32.0 µg), pressor effects mediated by peripheral vascular α_1- and α_2-adrenoceptors (Soc. Neurosci. Abs., 13: 1246). The latter observation was further investigated by measuring radioactivity in the blood, brain and spinal cord of rats after i.t. administration of [^{3}H]clonidine. Concentrations of clonidine (in ng/mg) in spinal cord and brain after i.t. injection of 32.0 µg [^{3}H]clonidine were:

TIME	Lumb. s.c.	Cerv. s.c.	Cerebel.	Front. ctx.	Pons-Medulla
5 min:	120.5±39.5	12.6±7.7	0.16±.04	0.17±.03	0.14±.04
20 min:	6.5±1.1	0.1±.01	0.09±.01	0.12±.01	0.08±.01

Concentrations of clonidine (in ng/ml) in the blood were:

DOSE	1 min	2 min	5 min	20 min
3.2 µg:	2.0±0.1	3.5±0.4	3.6±0.2	1.7±0.1
32.0 µg:	27.8±3.6	38.8±6.9	30.0±6.0	20.9±1.9

Administration of 2.5 µg/kg clonidine i.v. (sufficient to produce the peak blood levels observed after 32.0 µg i.t. clonidine) resulted in a transient pressor response. These results suggest that after i.t. administration of 32.0 µg clonidine, sufficient amounts of the drug are distributed systemically to produce peripherally mediated pressor effects.

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392.4

MECHANISM OF PRAZOSIN INDUCED SYMPATHO-INHIBITION. M.C. Koss, J.A. Hey and T. Ito*. Dept. Pharmacology, Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Intravenous administration of the alpha$_1$-adrenoceptor antagonist, prazosin (3-300 µg/kg), produced a depression of electrodermal sympathetic-cholinergic responses (EDRs) evoked by electrical stimulation of the posterior hypothalamus in pentobarbital anesthetized cats. Pretreatment with the alpha$_2$-adrenoceptor antagonists yohimbine (0.5 mg/kg) or idazoxan (0.1 mg/kg) significantly blocked the depressant effects of prazosin but had no effect on evoked EDRs when given alone. Electrodermal responses were readily elicited in animals depleted of CNS monoamines. Monoamine depletion, however, totally abolished prazosin's depression of centrally evoked EDRs. Similar results were obtained when EDRs were evoked by electrical stimulation of the cervical spinal cord in spinalized cats. These results suggest that prazosin produces its CNS sympatho-inhibitory effect by facilitation of inhibition mediated by alpha$_2$-adrenoceptor mechanisms and not directly by blockade of excitatory alpha$_1$-adrenergic receptors in the CNS. These findings also suggest a spinal cord site of action for prazosin. (Supported by the National Science Foundation)

392.5

RENAL CORTICAL AND MEDULLARY α_2 ADRENOCEPTORS ARE UPREGULATED BY HIGH NaCl DIET IN SHR-S. W. Sripairojthikoon* and J.M. Wyss. Dept. of Cell Biol. and Anat., Univ. of Alabama, Birmingham, AL 35294.

In NaCl sensitive, spontaneously hypertensive rats (SHR-S), a high NaCl diet elevates arterial pressure by decreasing central sympathoinhibition. This study tests the hypothesis that renal α_2 adrenoceptors in both cortex and medulla are upregulated following dietary NaCl loading; an effect that may exacerbate hypertension in SHR-S. In SHR-S the specific binding of the α_2 adrenergic agonist, [^{3}H] p-aminoclonidine (PAC [1 nM high affinity and 10 nM, low affinity]) is increased significantly 2 and 5 weeks after the initiation of a high (8%; compared to a basal, 1%) NaCl diet. In contrast, during the established phase of NaCl exacerbated hypertension (10 weeks), there are no differences in renal [^{3}H] PAC binding between diet groups. Parallel experiments using an α_2 adrenergic antagonist ([^{3}H] rauwolscine) confirm these results. The high NaCl diet does not affect arterial pressure or renal cortical or medullary α_2 adrenoceptors in normotensive, NaCl resistant Wistar-Kyoto rats (WKY) following 2 weeks on the diet, and the diet does not affect renal α_1 adrenoceptor binding in SHR-S or WKY. These findings indicate that in SHR-S, the NaCl induced upregulation of renal α_2 adrenoceptors may be independent of renal nerve activity, since both heavily innervated (cortical) and sparsely innervated (medullary) renal areas are affected equally.

392.6

TACTILE STARTLE IN SPONTANEOUSLY HYPERTENSIVE (SHR), BORDERLINE HYPERTENSIVE (BHR), AND WISTAR KYOTO (WKY) RATS IS UNAFFECTED BY GANGLIONIC BLOCKADE. C. H. Woodworth and A. K. Johnson. Departments of Psychology and Pharmacology, and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

Comparisons between SHR and WKY with respect to startle reactivity have yielded conflicting results. However, strain differences in startle reactivity may not be causally related to blood-pressure differences, since selective inbreeding can result in such traits being linked by chance. In the present study, tactile startle (TS) was assessed on two occasions, 7-9 days apart, in male, 17-week-old SHR, WKY and BHR (the F1 progeny of WKY♀ X SHR♂), with either ganglionic blocking agent or vehicle administered 20 min prior to the second test. Strain effects on TS were evident in the first (SHR>BHR=WKY), but not the second test. SHR showed a significantly greater test-retest decrease in TS than BHR or WKY, particularly at later trials and lower airpuff intensities. Hexamethonium (30 mg/kg, IA) produced a significant and equivalent drop in blood pressure across strains, but had no effect on TS. This suggests that startle reactivity is not sensitive to hypotensive changes produced by blocking sympathetic outflow to the periphery in these inbred strains.

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392.7

DIETARY TRYPTOPHAN INHIBITS DAHL SALT INDUCED HYPERTENSION. L. Lark*, P. Witt*, K. Becker*, W. Studzinski* and J. A. Weyhenmeyer. Neural and Behavioral Biology Program and College of Medicine, Univ. of Ill., Urbana, IL 61801.

Elevated dietary tryptophan (trp) has been found to block the development of DOCA/salt hypertension in rats (Fregley et al., Clin. Exp. Pharm. Physiol. 13: 767, 1986). We investigated the effect of such treatment on the inbred Dahl salt sensitive (DS/JR) rat, its normotensive controls, the inbred Dahl salt resistant (DR/JR) rat, and the outbred Sprague Dawley (SD) rat.

DS/JR animals placed on a supplemented trp diet (50 gm/kg food) at weaning had significantly lower pressures than those maintained on a diet with normal levels of trp (after 6 wks on a 1% salt diet, 123 ± 9 vs. 158 ± 23 mm Hg; after an additional 10 days on 8% salt, 147 ± 26 vs. 191 ± 28 mm Hg). Other groups showed no trp induced pressure differences at 6 wks or after 10 (SR/JR) or 14 days (SD) of salt loading. Slope coefficients were calculated for each animal and averaged within each group. For the initial 6 wk period the slope coefficient of the control DS/JR group was significantly greater than that of all groups on the high trp diet and when calculated over the entire course of the study was greater than all groups. Hearts of the control DS/JR rats were significantly larger than those of the same strain on the high trp diet. We conclude that elevated dietary trp protects against the development of hypertension and cardiac hypertrophy in DS/JR rats. Supported by NSF and AHA-IL.

392.8

CHANGES IN NOREPINEPHRINE (NE) MODULATION OF LATERAL HYPOTHALAMIC NEURONAL RESPONSIVENESS TO PUTATIVE NEUROTRANSMITTERS IN DOCA- HYPERTENSIVE RATS. F.M. Sessler and B.D. Waterhouse. Dept. of Physiol. & Biophys., Hahnemann Univ., Philadelphia, PA, 19102.

The lateral hypothalamus (LH) is involved in the central integration of fluid and electrolyte balance. Several studies have suggested a role for NE in these functions. In previous studies we presented evidence in support of a modulatory role for NE within the LH circuitry, i.e. NE was capable of facilitating responses of LH cells to synaptic inputs and putative transmitters. In the present studies we examined interactions of NE with LH neuronal responses to amino acid transmitters in the DOCA hypertensive rat, and compared the results with those from normal (ND) and high salt diet (HSD) rats. Male rats were uninephrectomized and received a DOCA implant (80mg, Dow Corning). They were given 1% NaCl in their drinking water (4-6 weeks). HSD rats received the same treatment but no DOCA. Extracellularly recorded responses from single LH neurons to iontophoretic pulses (5-55nA; 10-20 sec duration) of GABA or glutamate (GLU) were examined before, during and after NE microiontophoresis (5-50nA) in anesthetized rats. The results indicated a shift of NE modulatory action from potentiating to antagonizing effects such that in ND rats, NE routinely potentiated GABA depressant responses (33 of 46, 72%), whereas in HSD rats the ability of NE to enhance GABA responses was reduced to 31% of the cases tested (9 of 29). Likewise, NE did not augment but rather antagonized GABA inhibition in the majority of cells recorded (14 of 23, 61%) from DOCA hypertensive rats. NE action on GLU-induced excitation was also partially reversed from control (ND), in HSD and DOCA rats. In summary, these findings suggest that chronic HSD and DOCA treatments can alter the modulatory capacities of NE within the LH. Although many as yet undetermined mechanisms are likely to be responsible for such alterations, this plasticity of noradrenergic action may represent a central response to afferent synaptic signals which are coding for changes in salt intake and blood pressure.(Supported by AFOSR-87-0138 and NINCDS 18081 to B.D.W.).

392.9

ANALYSIS OF FACTORS PRODUCING AIR STRESS-INDUCED HYPERTENSION (HTN) IN THE BORDERLINE HYPERTENSIVE RAT (BHR). L.D. Fisher and A.K. Johnson. Departments of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, Iowa 52442.

The BHR is genetically predisposed to develop severe HTN if subjected to certain environmental stressors. We have reported previously that a stressor of intermittent, random jets of compressed air (AJS) directed to the head of BHR while mildly restrained in plexiglas holders (PGH) was effective in inducing HTN. In the present study we examined which of the component(s) (e.g., air jet, air noise, and/or restricted movement) of AJS in PGH was responsible for HTN. Six groups of BHR with comparable systolic blood pressures (SP; via tail cuff) were subjected to different experimental conditions for 2 hrs per day, 5 days per wk for 10 wks. After 10 wks, SP (mmHg) for the six groups were: (1) 189.8 ± 3.2 (n=10) for BHR receiving AJS directed toward the head while in PGH; (2) 187 ± 2.3 (n=7) for BHR receiving the noise of air but no air jet while in PGH; (3) 176.5 ± 2.5 (n=7) for BHR receiving AJS but while free to move about a cage like their home cage (CLH); (4) 164.7 ± 2.9 (n=7) for BHR placed in PGH without AJS: (5) 161.3 ± 2.4 (n=8) for BHR removed from their home cages and placed in CLH; and (6) 165.1 ± 1.5 (n=10) for BHR that served as maturation controls. The results indicate that in confined BHR air noise alone or in conjunction with tactile stimulation provides the most effective condition of those tested to produce HTN. In addition, it can be seen that air noise itself can be used without restraint to elevate SP in BHR.

392.10

LESIONS OF THE ANTERIOR HYPOTHALAMIC AREA (AHA) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR-S) DECREASE BAROREFLEX RESPONSE. R.M. Thornton*, R.H. Yang*, S. Oparil and J.M. Wyss, Departments of Medicine and Cell Biology and Anatomy and the Hypertension Research Program, University of Alabama, Birmingham, AL 35294.

Lesions of AHA chronically increase blood pressure in SHR-S but not in normotensive rats. To test whether alterations in baroreflex contribute to the hypertensive effect of AHA lesions, bilateral lesions of the AHA were made in 7 week old SHR-S. Three days after surgery, the lesion group displayed significantly elevated systolic arterial pressure compared to the sham-operated group. At 10 weeks of age (three weeks after the lesions were made) the lesion and control rats were instrumented with femoral, arterial and venous catheters, and 2 days later, the rats were volume loaded with whole blood (15% of each rats blood volume; .364 ml/min). Mean arterial pressures were 179 ± 4 and 163 ± 4 mm Hg ($p<0.01$) for the lesion and control rats, respectively. Neither group displayed a significant blood pressure response to the volume load. In contrast, the lesion SHR-S displayed a significant elevation of heart rate (HR; 31 ± 12 bpm, initial HR 377 ± 9 bpm) following the load, while sham operated SHR-S tended to slightly decrease heart rate following the load (12 ± 6 bpm, initial HR 396 ± 12 bpm). The results suggest that the AHA has an influence on baroreflex in SHR-S.

392.11

THE EFFECT OF ONE-KIDNEY GOLDBLATT HYPERTENSION ON ADRENERGICALLY-INDUCED THERMOGENESIS IN COLD ACCLIMATED RATS. J.R. Wilson[1] and D.M. Fyda[2], [1]Department of Psychology, University of Manitoba, Winnipeg, Manitoba R3T 2N2 and [2]Department of Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1.

The extension of certain thermogenic responses from cold to non-cold acclimated rats, along with the thermal consequences of renovascular hypertension were examined. Sixty-four male, Sprague Dawley rats were either cold or non-cold acclimated and rendered hypertensive (one-kidney, one-clip) or remained normotensive. Arterial and venous catheters were chronically implanted and the animals' metabolic, thermal and cardiovascular responses were assessed during the independent or simultaneous infusions of an α_1- or β_1-agonist. Overall, the α_1-agonist was non-thermogenic; whereas the β_1-agonist increased metabolism and body temperature. The simultaneous infusion of the α_1- and β_1-agonists potentiated the β_1-thermogenic effect. This overall response profile, although observed in the non-cold acclimated animals, was enhanced in the cold acclimated rats. Conversely, renovascular hypertension suppressed thermogenic reactivity to β_1-stimulation. The results suggest that the α_1-potentiation of β_1-induced thermogenesis is not restricted to the cold acclimated endotherm and that the reduced reactivity of the hypertensive animal may be linked to its purported state of hypothyroidism.

392.12

VASODILATORY EFFECT AND MECHANISMS OF CALCITONIN GENE RELATED PEPTIDE (CGRP) IN RESISTANCE VESSELS FROM NORMOTENSIVE AND GENETICALLY HYPERTENSIVE RATS. S.-P. Han* and T.C. Westfall (SPON: W.G. Wood). St. Louis University School of Medicine, St. Louis, MO 63104.

CGRP is known to exert cardiovascular effects. The current study was designed to investigate the effect and mechanisms of CGRP on resistance vessels from normotensive and hypertensive rats. Isolated mesenteric arterial bed from Sprague-Dawley (SD) or from 14 weeks old Spontaneously Hypertensive rat (SHR) was perfused and superfused. Perfusion pressure was continuously monitored and endogenous NE release was measured by HPLC-EC system. CGRP was given over a 5 min perfusion period. In preparations from SD rats, vasoconstriction induced by periarterial nerve stimulation (PANS) was inhibited by CGRP significantly. Both tonic and phasic contraction induced by exogenous NE ($5x10^{-5}$M) was inhibited by CGRP. 90% tension induced by NE was attenuated by 10^{-7}M CGRP. Endogenous NE release stimulated by PANS was not changed. The vasodilatory effect of CGRP on PANS induced vasoconstriction was significantly attenuated in the vascular beds from SHR compared to that from SD rats. Our results suggest that 1) CGRP is a potent vasodilator in mesenteric arterial vascular bed; 2) the postsynaptic mechanism contributes a major part of CGRP induced vasodilation; 3) the vasodilatory effect of CGRP is impaired in SHR which may help to explain the pathogenesis of hypertension. (Supported by HL26319 and HL35202.)

392.13

BRAIN STEM NORADRENERGIC TRANSMISSION IN PYRIDOXINE DEFICIENCY-INDUCED HYPERTENSION. M. Viswanathan*, C.S. Paulose*, Y.L. Siow*, and K. Dakshinamurti. Dept. of Biochemistry, Fac. of Medicine, Univ. of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

The synthesis of neurotransmitters dopamine and serotonin involves a pyridoxal phosphate-dependent decarboxylation step. We have demonstrated previously that pyridoxine deficiency in adult rats is associated with increased peripheral sympathetic activity leading to hypertension [Paulose et al. Hypertension 11(4) 1988]. The possibility that pyridoxine deficiency-induced hypertension results from altered noradrenergic transmission in the brain stem was investigated.

Adult male Sprague-Dawley rats (150-160 g) were fed either a pyridoxine-supplemented or pyridoxine deficient diet for eight weeks. The kinetic parameters of $alpha_2$ adrenoceptors in the brain stem was studied using p-[3,5-^{3}H] aminoclonidine ([^{3}H]-PAC). There was significant increase ($p < 0.01$) in the B_{max} of [^{3}H]-PAC binding to the high and low affinity $alpha_2$ adrenergic receptors in the brain stems of pyridoxine-deficient rats compared with that in control rats. The increase in B_{max} was not accompanied by any change in binding affinity. Pyridoxine deficiency also caused significant decrease in the maximal activity of dihydroxyphenylalanine (DOPA) decarboxylase in the brain stem (27% of control). These changes correlated with data on norepinephrine turnover in the brain stem of pyridoxine deficient rats. Our findings suggest that hypertension induced by pyridoxine deficiency may result from reduced alpha adrenergic output in the brain stem. (Supported by grants from Manitoba Heart Foundation and MRC of Canada)

392.14

STRESS-INDUCED ELEVATION OF PLASMA CATECHOLAMINES (CA) IN STROKE-PRONE HYPERTENSIVE RATS (SHRSP). PRC Howe*, PF Rogers*, X-F Zhou*+ and BG Livett+ CSIRO Divn of Human Nutrition, Adelaide, SA 5000 and + Biochemistry Dept, Univ of Melbourne, Carlton, VIC 3052, Australia.

Lowering blood pressure (BP) with the vasodilator, hydralazine, causes a greater reflex elevation of plasma CAs, esp. epinephrine (EPI), in SHRSP than in normotensive WKY (Howe et al, J Cardiovasc Pharm 8:1113, 1986). Since adrenal CA contents (unpublished results) and basal levels of plasma CAs are greater in SHRSP than in WKY, it was of interest to see whether plasma CA responses to other forms of stress are enhanced in SHRSP. In this study, effects of hypovolemic and hypoglycemic stresses on BP and plasma CAs were examined in conscious, unrestrained adult rats.

Initial BP readings and blood samples were taken at rest via an indwelling arterial catheter. To induce hypovolemia blood was withdrawn at a constant rate of 2.5ml/min. Mean BP fell after 20 min from 113 to 44 in WKY (n=4) and from 167 to 75 mm Hg in SHRSP (n=5). Plasma EPI rose from 180 to 3600 and from 840 to 5200 pg/ml in WKY and SHRSP resp. The corresponding rise of plasma norepinephrine (NE) was less than twofold. Most of the rise in EPI had occurred in SHRSP by 10 min but was unchanged in WKY at that stage.

Other rats were made hypoglycemic by i.v. administration of insulin and somatostatin. Results appear in the table.

Treatment time:	0 min		15 min		30 min	
(n=5 in each case)	WKY	SHRSP	WKY	SHRSP	WKY	SHRSP
PLASMA NE (pg/ml)	360	670	1190	1650	1540	2830
PLASMA EPI (pg/ml)	110	770	5380	7440	5500	15150
PLASMA GLUCOSE (mM)	5.9	6.7	1.8	1.9	2.8	2.1

BP was not affected by the treatment. The increases of CAs after 30 min were reversed by ganglion blockade.

The results confirm that the resting level of plasma EPI is substantially higher in SHRSP than WKY and that EPI is released preferentially in response to both metabolic as well as circulatory stress. They suggest that adrenal medullary function may be selectively augmented in SHRSP.

392.15

CIRCADIAN RHYTHM OF ATRIAL NATRIURETIC PEPTIDE IS ABSENT IN HUMAN AUTONOMIC FAILURE. H.C.Kaufmann*, A.R. Pierotti*, J.L.Roberts, and M.D.Yahr. (SPON: T Elizan) Dept of Neurology and Fishberg Center for Neurobiology, Mt. Sinai Sch. of Med., CUNY, New York NY 10029.

Orthostatic hypotension in autonomic failure is due to defective norepinephrine release and exaggerated renal sodium loss, especially at night during recumbancy. The exact mechanism responsable for this defect is not known. Atrial natriuretic peptide (ANP) is a recently discovered hormone that causes natriuresis and relaxation of smooth muscle. To determine the role of ANP in the renal sodium wasting of patients with autonomic failure, the peptide was measured in patients with AF and in age matched control subjects. All subjects were in sodium balance. Blood samples were collected during a 24hr period at 4 hourly intervals with the patients recumbent.

The mean daily plasma concentration of ANP in patients with autonomic failure was 45±6pg/ml and in controls 57±4pg/ml (mean±sem). Control subjects showed significantly higher levels of ANP during the night than during the day. In contrast, patients with autonomic failure exhibited no significant variation in the concentration of ANP throught the day. ANP, however, responded appropriately to changes in extracellular fluid volume induced by the administration or withdrawl of fludrocortisone in patients with autonomic failure.

Since ANP is not increased in autonomic failure, it is unlikely that abnormalities in its secretion are responsible for the salt wasting and low blood pressure seen in these patients. Whether the blunted circadian rhythm of ANP secretion is primary or secondary to the autonomic failure remains to be elucidated.

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392.16

NEUROPEPTIDE Y PRODUCTION IN RAT ATRIAL MYOCYTE CULTURE. K.L.Marek and R.E.Mains, Depts. Neurology and Neurosci., Johns Hopkins Univ. School of Medicine, Balt. Md 21205.

We have examined Neuropeptide Y (NPY) production in primary atrial myocyte cultures form neonatal rats. NPY content was measured in cell extracts and in culture medium by gel filtration followed by radioimmunoassay. Exogenous NPY was stable in the culture medium for at least 48 hours. NPY mRNA in cell extracts was quantitated by Northern analysis.

Atrial myocyte dissociated cell cultures were maintained in complete serum free medium for up to 3 weeks. NPY-immunoreactivity was identified in atrial myocytes in cultures using the avidin-biotin/peroxidase complex method. Spent culture medium collected over 48 hours contained 0.9 pmole NPY/atrial equivalent. NPY content was reduced 5-fold when cultures were grown in medium containing 100 nM dexamethasone or 100 μM dBcAMP and increased 2-fold by growth in medium containing 100 nM phorbol myristate acetate (PMA). NPY mRNA by Northern analysis showed similar changes in response to these treatments.

Thus cultured rat atrial myocytes produce and secrete NPY. The production of NPY can be regulated in culture by distinct second messenger systems. Experiments are underway to examine further the regulation of NPY production in atrial myocytes. Support: NS01168, DA-00266, DA-00097.

392.17

CHARACTERISTICS OF NERVES, NODAL CELLS AND ANF GRANULES IN HEARTS FROM STZ DIABETIC RATS. A.B. Drakontides and M.G. Dominguez*, Department of Anatomy, New York Medical College, Valhalla, NY 10595.

Cardiac disease is commonly present as a complication in chronic diabetes. The streptozotocin (STZ) induced diabetic rat was used as an experimental model to study ultrastructural features of heart tissue. Analysis of nerve bundles in the vicinity of SA and AV nodes indicated that myelinated nerve fibers had the greatest number of aberrations; unmyelinated fibers were either normal or had disrupted axoplasm, increased numbers of vacuoles and myelin whorls. Compared to normal hearts Type P nodal cells in diabetic hearts appeared to have increased numbers of vacuoles and residual bodies; mitochondria often had disrupted cristae. Atrial cardiocytes were significantly smaller in diabetic (41.45 μm^2 ± 5.07) as compared to control (143.25 μm^2 ± 31.04). This appeared to be related to the degree and duration of the diabetic state. Average numbers of atrial granules per area of cell were similar in diabetic and controls. In control hearts, atrial granules were usually of the same electron density; in diabetics, a greater variation in electron density was noted with residual bodies commonly intermingled among granules, furthermore, granules were more frequently distributed closer to cell surfaces. This morphology may be indicative of functional alterations in synthesis, storage and release of natriuretic peptides (ANF) in the diabetic state.

393.1

CHRONIC EXPOSURE TO RO 15-1788 DECREASES GABA FACILITATION OF [3H]FLUNITRAZEPAM BINDING IN RAT NEOCORTICAL MEMBRANES. M. Urbancic* and T.J. Marczynski. Dept. of Pharmacology, Univ. of Illinois, College of Med., Chicago, IL. 60612.

We have investigated the effect of chronic Ro 15-1788 treatment on GABA facilitation of [^{3}H]Flunitrazepam ([3H]FNTZ) binding in neocortical and hippocampal membranes from Ro 15-1788-treated (4mg/kg/day for 14 days in drinking water) and vehicle-treated, 3 months old Wistar rats. The rats were sacrificed 72 hrs following Ro 15-1788 withdrawal. Binding assays were performed in the absence or presence of GABA (10μM) and NaCl (200 mM). Scatchard analysis showed that GABA increased the affinity and the density of benzodiazepine (BDZ) binding sites:

	CONTROL		Ro 15-1788	
	HIPP.	CORTEX	HIPP.	CORTEX
Kd	21.7% ss	18.3% ss	15.3% ss	13.3% ss
Bmax	7.6% s*	11.1% s*	5.4% s	3.7% s

s=p<.04; s*=p<.01; ss=p<.005; paired t-test.

The GABA enhancement of [3H]FNTZ binding was reduced by 67% (p<.02) in neocortical membranes from Ro 15-1788 treated rats, as compared to the control group. Thus, chronic exposure to a BDZ receptor antagonist, Ro 15-1788, has a prolonged effect on the coupling between the BDZ and GABA components of the receptor complex.

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393.2

DOWNREGULATION OF $GABA_A$ RECEPTOR FUNCTION AFTER CHRONIC CLONAZEPAM TREATMENT IN PRIMARY NEURON CULTURES. R.B. Roy* and L.G. Miller(SPON: L. Happel). Depts. of Medicine and Pharmacology, LSU Medical Ctr., New Orleans, LA 70112.

Chronic benzodiazepine administration has been reported to decrease binding and function at the $GABA_A$ receptor complex in intact animals. To assess the effects of chronic benzodiazepines in a controlled setting, we administered clonazepam, 0.1, 1, and 10 uM, to chick cerebral neurons in primary culture. $GABA_A$ receptor function was assessed by muscimol-stimulated uptake of $^{36}Cl^-$ as described by Thampy and Barnes(J. Biol. Chem., 259:1753, 1984). All assays were performed after 10 days of culture. Chloride uptake in cells treated with vehicle alone for 10 days was similar to controls. Uptake after clonazepam, 1 uM, for 2 and 4 days was similar to controls, and there was a trend toward increased uptake after 6 days. After 10 days, uptake was markedly decreased at all doses of clonazepam evaluated. Chloride uptake was also unchanged compared to controls after 2 days of clonazepam, 0.1 and 10 uM, but in both cases was substantially decreased at 10 days. These results in cultured neurons corroborate reports of decreased $GABA_A$ receptor function in animals, and indicate that downregulation in cultured cells occurs at physiologically relevant drug concentrations.

393.3

DIFFERENTIAL EFFECTS OF ZINC ON VERTEBRATE NEURONAL $GABA_A$ RECEPTORS. T.G. Smart* and A. Constanti* (SPON: M.J. Neal). MRC Neuropharmac. Res. Gp., Dept of Pharmacology, London University, School of Pharmacy, London U.K.

Zinc is an abundant ion in the mammalian central nervous system where it is concentrated into discrete areas including the hippocampus and the cortex. On cultured rat superior cervical ganglion neurones using patch clamp recording, 10-100μM zinc resulted in a reversible inhibition of GABA-evoked membrane current responses. This inhibition was more potent in neurones derived from foetal rats compared to cultures prepared from post-natal and adult animals. However using intact, adult rat sympathetic ganglia, zinc was virtually ineffective as a GABA antagonist. In contrast, intracellular recording from brain slices of hippocampus and pyriform cortex revealed zinc (100-500μM) as a powerful and reversible enhancing agent of GABA responses. This enhancement had a long latency (~30 mins) and was not due to blockade of GABA uptake since it occurred in the presence of 1mM nipecotic acid. These results suggest that zinc has a differential effect on $GABA_A$ receptors which may be dependent on the type of preparation and possibly also on the stage of neural development.

This work is supported by the Medical Research Council.

393.4

CONVULSANT INSECTICIDES POTENTIATE THE PROTECTIVE EFFECT OF NaCl AGAINST HEAT INACTIVATION OF ^{3}H-FLUNITRAZEPAM BINDING SITES. R.F. Squires and E. Saederup.* Nathan Kline Inst., Orangeburg, N.Y. 10962.

Earlier, we reported that picrotoxin and several "cage" convulsants potentiated the protective effect of NaCl against heat inactivation (60°C, 30 min.) of ^{3}H-Flunitrazepam binding sites on rat brain membranes. We now report that a series of polychlorinated convulsant insecticides, all of which potently inhibit the binding of ^{35}S-TBPS to rat brain membranes, also potentiate the protective effect of NaCl against heat inactivation of ^{3}H-flunitrazepam binding sites with a rank-order or potencies which is similar (but not identical) to the rank-order of potencies for inhibition of ^{35}S-TBPS binding. The most potent protector was α-endosulfan (ΔB_{max}=26%; EC_{50}=120 nM) followed by endrin (39%, 0.20 μM), heptachlorepoxide (24%, 0.96 μM), dieldrin (25%, 1.3 μM), toxaphene (35%, 1.5 μM) and lindane (25%, 3.1 μM). As inhibitors of ^{35}S-TBPS binding, the rank-order of potencies was as follows: α-endosulfan (IC_{50} = 11 nM), endrin (12 nM), dieldrin (130 nM), heptachlorepoxide (170 nM), toxaphene (185 nM) and lindane (210 nM). These results provide further confirmation of a direct molecular coupling of picrotoxin (TBPS) and benzodiazepine (flunitrazepam) binding sites in the GABA-A receptor complex.

393.5

REGULATION OF THE PICROTOXININ RECEPTOR BY ALKYL SUBSTITUTED γ-BUTYROLACTONES AND γ-THIOBUTYROLACTONES. K.D. Holland*, A.C. McKeon*, S.M. Rothman, D.F. Covey*, and J.A. Ferrendelli Depts. of Pharmacology, Neurology, and Anatomy and Neurobiology, Washington Univ. Medical School, St. Louis, MO 63110.

Alkyl substituted γ-butyrolactones (GBLs) and γ-thiobutyrolactones (TBLs) exhibit convulsant or anticonvulsant activity depending on the location of the alkyl substituents. We examined the effects of a convulsant agent, β-ethyl-β-methyl-TBL (β-EMTBL), and two anticonvulsants, α-ethyl-α-methyl-GBL (α-EMGBL) and α-ethyl-α-methyl-TBL (α-EMTBL) on binding of ^{35}S-t-butylbicyclophosphorothionate (TPBS), a ligand specific for the picrotoxinin receptor, and on GABA-mediated currents in cultured rat hippocampal neurons. Beta-EMTBL, α-EMTBL, and α-EMGBL all competitively inhibited ^{35}S-TBPS binding in a dose dependent manner, with IC_{50}s of 9μM, 365μM, and 2.3 mM respectively. In voltage clamped neurons, the convulsants, β-EMTBL and picrotoxinin, inhibited GABA-mediated currents; the broad spectrum anticonvulsant, α-EMTBL, augmented GABA-mediated currents; and α-EMGBL, an anticonvulsant with a limited spectrum of action, had no effect. However, α-EMGBL blocked both the inhibitory effect of picrotoxinin and the facilitory effect of α-EMTBL. Thus, the present results indicate that all three of the above compounds can regulate chloride conductances probably by acting at the picrotoxinin receptor. It appears that each has a different effect. We propose that β-EMTBL is an agonist; α-EMGBL is an antagonist and α-EMTBL is an inverse agonist.

Supported by NIH Grant GM07805 and the Seay Neuropharmacology Research Fellowship.

393.6

BIOCHEMICAL CHARACTERIZATION OF INSECT GABA-A RECEPTORS. J.J. Rauh* and E.A. Benner* (SPON: J. Meek). Agric. Prod. Dept., E.I. du Pont de Nemours & Co., Wilmington, DE 19898.

Pharmacological differences between insect and vertebrate GABA-A receptors suggest that multiple subtypes of GABA-A receptors may exist. Our studies on the GABA-dependent chloride channel of housefly thorax have revealed additional differences between insect and vertebrate receptors.

Addition of sulfhydryl reducing agents to membrane preparations from housefly thorax increased binding of 35S-TBPS, but had no effect on rat brain membranes. N-ethylmaleimide treatment eliminated 35S-TBPS binding to housefly membranes (IC50=10 μM) but did not alter binding to rat membranes. These results suggest sulfhydryl groups play a role in determining binding interactions at the insect TBPS site.

Insect and vertebrate receptors also differed in their interactions with steroidal compounds which potentiate chloride channel opening. Although we found these steroids to be potent allosteric displacers of 35S-TBPS from rat membranes (IC50=10 nM-1 μM), they were 100-1000 fold less active on housefly membranes. Since steroid displacement of 35S-TBPS was by an allosteric mechanism, we investigated steroid interactions at the ivermectin binding site but found no displacement of 3H-ivermectin from either rat or housefly membranes. This suggests that steroids interact with a unique binding site on GABA-A receptors. We are currently assessing the effects of these steroids, as well as sulfhydryl reagents, on GABA-A receptors of identified neurons from cockroach.

393.7

MOLECULAR HETEROGENEITY OF THE GABA/BENZODIAZEPINE RECEPTOR COMPLEX. D.PARK*, J.Vitorica* and A.L.de Blas. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

Recently, we have reported the production of monoclonal antibodies (mAbs) to the GABA/benzodiazepine receptor (GABAR/BZDR) complex (Vitorica et.al., J. Neurosci.,8:615-622,1988). The mAb 62-3G1 recognized the 57,000 Mr polypeptide of the GABAR/BZDR complex in immunoblots. Immunohistochemistry using this antibody revealed the colocalization of mAb 62-3G1 immunoreactivity and the [^{3}H] muscimol binding sites (De Blas et.al., J. Neurosci.,8,601-614, 1988). It has been proposed that there are subtypes of benzodiazepine receptors with differential distribution in various brain areas. This notion is supported by differences in ligand binding and photoaffinity labeling in various areas of the brain.

In this communication, we are investigating the molecular basis of the heterogeneity in GABAR/BZDR complex. For this purpose, we have used the immobilized mAb 62-3G1 for the purification of GABAR/BZDR complex by immunoaffinity chromatography from both the bovine cerebral cortex and cerebellum. The GABAR/BZDR complex, either photoaffinity labeled or not with [^{3}H] flunitrazepam, was solubilized from bovine cerebral cortex or cerebellum and applied to the mAb 62-3G1 Affi-gel 10 column. The SDS-PAGE and silver staining of the immunoaffinity purified receptor, revealed polypeptides of 51,000, 55,000 and 57,000 Mr in cortex, but only 51,000 and 57,000 Mr polypeptides were apparent in cerebellum.

393.8

DIFFERENTIAL DISTRIBUTION OF $GABA_A$/BENZODIAZEPINE RECEPTOR SUBUNIT mRNAs IN THE BOVINE CEREBELLUM. R.E. Siegel, Dept. of Pharmacology, Case Western Reserve Univ., Cleveland, OH 44106.

The major inhibitory neurotransmitter in the brain, γ-amino butyric acid (GABA), acts at specific neuronal receptors. In addition to GABA, the receptor possesses a binding site for the benzodiazepines, a class of anxiolytics. This $GABA_A$/benzodiazepine receptor complex is composed of α and β subunits to which the benzodiazepines and GABA bind, respectively. While the receptor is generally believed to have a uniform subunit composition throughout the brain, autoradiographic studies with GABA ligands suggest structural heterogeneity in the cerebellum.

To examine further the expression of the $GABA_A$/benzodiazepine receptor in the bovine cerebellum, we have used in situ hybridization histochemistry. Oligonucleotide probes complementary to the mRNAs encoding the α and β subunits of the bovine receptor (Schofield et al, Nature 328: 221, 1987) were chemically synthesized and labeled with ^{35}S. Following hybridization and autoradiography, a uniform distribution of grains was detected over the granule cell layer with probes for both subunits. Intense labeling was also observed over the Purkinje and molecular cell layers with the α subunit probe. In contrast, no β subunit mRNA was detectable in these regions. As cells in these layers respond to GABA, our findings suggest that additional, as yet unidentified, β subunits exist. (Supported by NIH Grant MH42173 and the Mathers Foundation)

393.9

DIFFERENTIAL EFFECT OF R015,1788 ON [^{3}H]-FLUNITRAZEPAM AND [^{3}H]-β-CARBOLINE BINDING ON RHESUS MONKEY BRAIN SECTIONS. J. B. O'Neill[1], J.M. Crawley[2], and D.P. Friedman[3,4]. [1]Section on Brain Biochemistry and [2] Clinical Neuroscience Branch, and [3]Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda, MD 20892, and [4]Division of Preclinical Research, NIDA, Rockville, MD 20857

Biochemical evidence suggests that benzodiazepine (BDZ) and β-carboline (BC) binding sites are part of a supramolecular complex. In support of this, we presented (O'Neill *et al.*, Soc. Neurosci. Abstr. 10(1):552, 1984) anatomical evidence for the co-localization of [^{3}H]-Flunitrazepam (FLU) and [^{3}H]-BC binding sites at the light microscopic level. To extend this analysis and to determine if these two drugs are binding to the same molecular site, we performed competition studies using bicuculline (BIC), diazepam (DZ), RO15,1788 (RO) in μM amounts to displace nM amounts [^{3}H]-FLU and [^{3}H]-BC on slide mounted rhesus monkey brain sections. Three monkeys were deeply anesthetized by intrahepatic injection of sodium pentothal and the brains quickly removed. 25 μM sections were prepared as described previously (Bachevalier *et al.*, Develop. Brain Res. *25*:302-308, 1985).

Binding characteristics were determined for [^{3}H]-FLU and [^{3}H]-BC and incubations took place at their K_ds. Bic did not alter binding under any of these conditions, suggesting that only ineffective concentrations of endogenous GABA remained in these sections

RO and DZ differentially blocked the binding of [^{3}H]-FLU and [^{3}H]-BC, whereas the binding of [^{3}H]-FLU was amost completely blocked by RO and DZ, [^{3}H]-BC binding was only partially inhibited by these compounds.

These differential effects suggest that [^{3}H]-FLU and [^{3}H]-BC are binding to different sites on the GABA/BDZ complex.

393.10

$GABA_A$ RECEPTOR COMPLEX PHARMACOLOGY IN RAT BRAIN SYNAPTONEUROSOMES. T. M. DeLorey, G. B. Brown. The Neuropsychiatry Research Program and the Dept. of Chem., Univ. of AL at Birmingham, Birmingham, AL 35294.

The physiology of the $GABA_A$ receptor complex is being reevaluated under more pysiological conditions with the intention of relating binding efficacy to funtional activity. Ligand binding studies of the $GABA_A$ receptor site are typically done with freeze/thawed synaptic membranes incubated at 0°C in 50 mM Tris-Citrate buffer. Dissociation constants for GABA agonists determined by this method are in the low nanomolar range. This is puzzling because micromolar concentrations are required to elicit a physiological response in vivo. These micromolar concentrations are also seen in $^{36}Cl^-$ flux studies done in vitro. To try to better understand what is occuring in the living system, binding assay conditions have been modified to better reflect the "native" state of the receptor in the living cell membrane. This new method employs the use of synaptoneurosomes containing both pre and post synaptic vesicular structures that have the ability to maintain a membrane potential . In addition, the buffer used in this new approach is more physiologically relevant. The buffer contains 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.18 mM $MgSO_4$ and 20 mM HEPES/9 mM Tris base (pH 7.4). The incubation period is 25 min. at a temperature of 22°C. This is contrasted with the standard assay incubation temperature of 0°C for 5 min. Incubation is terminated by dilution with ice cold buffer followed by rapid filtration through Whatman GF/C filter paper using a Brandel Cell Harvester. The tissue on the filter is washed twice with approximately 2.5 ml ice cold buffer. Data are analyzed by equilibrium dose response curves and Scatchard analysis using a best fit model (LIGAND).

The K_D values as determined by the standard binding assay show GABA to have two binding affinities with K_D's of 10 nM and100 nM. For muscimol there is only a single site with an K_D of 5 nM. $^{36}Cl^-$ flux studies have demonstrated EC_{50} values for GABA and muscimol of 10μM and 3μM respectively. These values are compared to the K_D values determined in the new binding assay protocol, which reveal GABA to have a K_D of 6 μM and muscimol to have a value of 400 nM. The antagonist (+)bicuculline and the agonists 3-aminopropane sulfonic acid and imidazolacetic acid have also been tested by this new binding method and correlate well with the physiologically relevant dose. Allosteric interactions among the $GABA_A$ receptor complex ligands in this new system compared to the standard system will be discussed.

393.11

BOVINE $GABA_A$ RECEPTOR SEQUENCE-SPECIFIC POLYCLONAL ANTIBODIES. F.A. Stephenson* and M. J. Duggan* (SPON: D. Bousfield) MRC Molecular Neurobiology Unit, Hills Rd, Cambridge CB2 2QH, England.

The primary structures of bovine $GABA_A$ receptor α1 and β subunits have been deduced from the corresponding complementary DNAs (Schofield et al, Nature, 1987, 328, 221-227). We have synthesised five peptides of length 13-17 amino acids which are contained within the α1 and the β polypeptide sequences. These peptides were glutaraldehyde-conjugated to keyhole limpet haemocyanin and used as antigen in the production of sequence-specific polyclonal antibodies. Antibody production was monitored by an enzyme linked immunosorbent assay (ELISA) with the respective immobilised peptide as antigen, followed by ELISA with purified bovine $GABA_A$ receptor as antigen. In all cases, antibodies against peptides were obtained but to date antibodies to three different sequences of the α1 subunit showed reactivity with the receptor in the ELISA assay. It was shown that the sera raised from each of these three antigens reacted with a 53000 (α) polypeptide in Western blots. Additionally, each serum immunoprecipitated specifically GABA and benzodiazepine binding sites from purified preparations and detergent extracts of cerebral cortical $GABA_A$ receptor. Thus we have identified three immunogenic regions of the $GABA_A$ receptor and antibodies directed at these epitopes recognise the receptor in its native and denatured form.

393.12

ENHANCEMENT OF FLUNITRAZEPAM BINDING BY PENTOBARBITAL: AN AUTORADIOGRAPHIC STUDY. H.A. Baghdoyan and R.A. Hawkins. Department of Anesthesia, The Penn. State University, College of Medicine, Hershey, PA 17033.

Previous studies using brain homogenates have demonstrated that pentobarbital enhances the binding of benzodiazepines (BDZ) in the cortex. The purpose of the present study is to investigate whether pentobarbital-induced enhancement of BDZ binding shows regional specificity throughout the brain. Such specificity might help elucidate how barbiturates induce their hypnotic effects. Thin sections of fresh-frozen rat brain were incubated with 3H-flunitrazepam or 3H-flunitrazepam plus pentobarbital and processed for film autoradiography. Various regions were measured throughout the brain, and all regions showed increased 3H-flunitrazepam binding in the presence of pentobarbital. These increases averaged 18% and ranged from 4% to 59%. The greatest increases observed were in the central gray (59%), the occiptal cortex (27%), the insular cortex and the occulomotor complex (24%). Adjacent sections, with and without pentobarbital, have been digitized. Comparison of these images will yield a point-by-point map of the influence of pentobarbital on flunitrazepam binding throughout the brain.

Supported by the Department of Anesthesia and a Research Initiation Grant, The Penn. State University.

393.13

BOTH γ-AMINOBUTYRIC ACID AND BENZODIAZEPINE LIGANDS PHOTOAFFINITY LABEL BOTH α AND β SUBUNITS OF THE GABA RECEPTOR. M. Bureau* and R.W. Olsen (SPON: D.J. Jenden). Department of Pharmacology, School of Medicine, and Brain Research Institute, University of California, Los Angeles, CA 90024.

The γ-aminobutyric acid(GABA)/benzodiazepine(BZ)receptor protein from bovine, rat, and human brain was purified by affinity column (Stauber et al., Eur.J.Biochem. 167, 125-133, 1987) and photoaffinity labeled with (^{3}H)muscimol and (^{3}H)flunitrazepam(FLU), followed by SDS gel electrophoresis. The BZ ligand (^{3}H)FLU was incorporated primarily into the expected 52 kD α subunit, but also significantly (60% of α in rat) into the 57 kD β subunit. Likewise, the GABA ligand (^{3}H)muscimol was incorporated primarily into the 57 kD β subunit, but also significantly (38% of β in human) into the 52 kD α subunit, suggesting that both major subunit peptides carry binding sites for both classes of ligands. Photolabeling was totally prevented by including excess cold ligand, and other peptides present (trypsin inhibitor) were not labeled. Further, both subunits α and β showed doublets for photolabeling with both ligands in all three species of animal, consistent with receptor subtypes possibly a family of gene products (Bureau & Olsen, FASEB J. 2, A622, 1988, #1892). The pharmacological specificity and brain regional distribution of the two α and β subunits were determined and are consistent with receptor subtypes.

Supported by NIH grants NS 22071 and NS 21908.

393.14

EFFECTS OF COCAINE ON BENZODIAZEPINE RECEPTOR LABELING IN VIVO. N. E. Goeders and M. A. McNulty*. Department of Pharmacology & Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130.

The chronic administration of cocaine has been reported to differentially affect a variety of neuronal systems including benzodiazepine receptor densities measured in vitro in various regions of the rat brain (Goeders et. al., Soc. Neurosci. Abst., 13:724, 1987). This investigation was therefore designed to determine the effects of cocaine on these receptors using in vivo receptor labeling procedures.

Adult male rats were injected via chronically implanted jugular catheters with 25 uCi [^{3}H]Ro 15-1788 5 min following pretreatment with cocaine (5, 10, 20 or 40 mg/kg, ip) or saline (1 ml/kg). The rats were sacrificed by decapitation 10 min following the intravenous injection, the brains were rapidly removed and dissected into cerebral cortex, hippocampus, striatum, cerebellum, diencephalon and brain stem, and radioactivity was directly determined in each tissue sample using liquid scintillation spectrophotometry. Blank values were determined by pretreating the animals with clonazepam (5 mg/kg, ip) 30 min prior to the intravenous injection. The results of this study will be discussed in relation to those obtained in vitro. [Supported in part by USPHS grant DA04293.]

393.15

ISOLATION OF cDNAs CLOSELY RELATED TO GABA-A RECEPTOR POLYPEPTIDES. M. Khrestchatisky[1*], A.J. MacLennan[1*], R.W. Olsen[2,3], M. Bureau[2*] and A.J. Tobin[1,3,4]. Departments of [1]Biology and [2]Pharmacology, [3]Brain Research Institute, and [4]Molecular Biology Institute, University of California, Los Angeles, CA 90024-1606.

Our work addresses the molecular composition and heterogeneity of the GABA-A receptor. We have prepared oligonucleotide probes (derived from the sequences of Schofield et al., Nature 328:221-227, 1987) for GABA-A receptor cDNAs and have used them to isolate candidate cDNAs for GABA-A receptor polypeptides.

We isolated several independent clones that hybridize with alpha subunit sequences. These were part of a cDNA library in lambda-gtll originally derived from bovine cerebral cortex mRNA by Dr. Rachel Neve. They have 70-80% identity with the published bovine alpha cDNA sequence. Under hybridization conditions of medium stringency, these probes detect genomic restriction fragments totaling 12-20 kb in the DNA of cows, rats, and humans.

We are presently extending these studies to the genes encoding other GABA-A receptor polypeptides.

This work was supported by grants to AJT from NINCDS (#NS22256 and NS20356), the Scottish Rite Schizophrenia Research Program and a program project grant to Dr. A.V. Delgado-Escueta (#NS21908). AJM was supported in part by a Canadian MRC Fellowship, and MK by a fellowship from the Fondation de l'Industrie Pharmaceutique pour la Recherche.

393.16

PURIFICATION OF A MITOCHONDRIAL BENZODIAZEPINE BINDING SITE PROTEIN. L. Antkiewicz-Michaluk*, A.G. Mukhin*, A. Guidotti, and K.E. Krueger. FGIN, Georgetown Univ. Med. Sch., Washington, D.C. 20007.

The photoaffinity probe [^{3}H]PK 14105 was employed to photolabel peripheral-type benzodiazepine binding sites on adrenal mitochondrial membrane fractions. The membranes were solubilized in 1% digitonin and the detergent-soluble extract was subjected to anion-exchange chromatography and reversed-phase high pressure liquid chromatography. This scheme resulted in the purification of a protein covalently modified by [^{3}H]PK 14105 and possessing an apparent molecular weight of 17 kilodaltons as determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis. Electrophoretic analysis of purified preparations of this protein revealed a single band following radioiodination with Bolton-Hunter reagent and also upon silver-staining of the gel. Amino acid analysis of the purified protein predicted a minimal molecular mass of approximately 16 kilodaltons. Cyanogen bromide digestion produced peptide fragments which were isolated and sequenced indicating that this protein shows no significant homology with other known protein sequences. These results demonstrate that a putative protein component of peripheral-type benzodiazepine recognition sites has been purified to apparent homogeneity and partially characterized.

393.17

A MOLECULAR CHARACTERIZATION OF THE γ-AMINOBUTYRIC ACID (GABA) RECEPTOR PROTEIN AND mRNA FROM RAT HIPPOCAMPUS DURING DEVELOPMENT. P. Gallombardo, N. Saito*, R. Duman, and J. Tallman, Abraham Ribicoff Research Facilities, Depts. of Pharm. and Psychiatry, Yale Univ. School of Med., 34 Park St., New Haven, CT 06508. K. Garrett and M. Vitek, Lederle Laboratories, Pearl River, NY.

The GABA receptor is composed of at least two subunits (α and β) and contains the binding sites for GABA, the anxiolytic benzodiazepines (BZ), and the anxiogenic β-carbolines. Studies of benzodiazepine binding have suggested the possible existence of multiple receptor "subtypes" or isoreceptors in the rat hippocampus and other brain regions. Photolabeling studies carried out during development have shown the existence of multiple proteins that can be labeled with BZ ligands. Recently, monoclonal antibodies with relative specificity for subunits of the GABA receptor have been raised and a human cDNA clone for the α subunit has been identified (see K. Garrett et al., this issue). Characterization of these subunit specific monoclonal antibodies and cDNA probes will be presented. The existence of multiple GABA receptor subtypes and mRNA in the rat hippocampus during development has been followed using photolabeling, immunobloting, and northern blotting techniques. The relationship of multiple labeled species and genes coding for them will be presented.

393.18

AN IN-VITRO STUDY OF THE EFFECT OF UNSATURATED FATTY ACIDS ON THE GABA/BENZODIAZEPINE RECEPTOR COMPLEX. M.R. Witt* and M. Nielsen. Psychopharmacological Research Lab., St. Hans Hospital, DK-4000 Roskilde, Denmark.

In our search for endogenous modulators of the GABA/benzodiazepine receptor complex (GBRC), we isolated oleic, arachidonic and docosahexaenoic acids from pig brain. At concentrations of 10^{-7}M to 10^{-4}M, these compounds enhance the specific binding of benzodiazepine receptor agonists to rat membranes in-vitro via an increase in affinity (K_D effect).

The stimulation of the binding of benzodiazepine receptor agonist is additional to the binding enhancement of these compounds induced by GABA. The binding of antagonist ([^{3}H]RO-151788) and inverse agonist ([^{3}H]DMCM) is only slightly stimulated. The number of binding sites labelled by [^{3}H]muscimol is increased 2-3 times (B_{max} effect), while the binding of [^{3}H]SR-95333 is unchanged. Pretreatment of the membranes with $AgNo_3$ or incubation with phospholipase A_2 abolish these effects of the fatty acids on the GBRC.

The binding of [^{35}S]TBPS, a ligand that labels sites closely related to the chloride ionophore, is inhibited by the fatty acids in a chloride-concentration-dependent manner. Whether unsaturated fatty acids have any physiological relevance in the regulation of GBRC is unknown.

393.19

A POSSIBLE ROLE FOR PHOSPHORYLATION IN THE MAINTENANCE OF $GABA_A$ RECEPTOR FUNCTION.

M. Gyenes*, M. Farrant*, and D. H. Farb. (SPON: B. M. Altura) Department of Anatomy and Cell Biology, SUNY Health Science Center, Brooklyn, New York 11203.

When using whole-cell recording methods we have observed a time-dependent decline in the responsiveness of cultured chick spinal cord neurons to the neurotransmitter GABA. In the present study we have investigated the role of ATP in this phenomenon. Cells were voltage-clamped at their resting potential (-55 to -70mV) and responses to pressure applied GABA (15-25s) were obtained at 10 minute intervals. Intracellular Ca^{2+} was buffered with EGTA (11mM). The peak amplitude of the inward current evoked by 3μM GABA (~200pA) was stable for up to 60 minutes. In contrast, the response to 30μM GABA (~2nA) progressively declined to approximately 30% of its initial value after 30 minutes. Similar results were obtained when these two concentrations of GABA were applied in sequence to the same cell. "Run-down" of the response to 30μM GABA was reduced when 5mM Mg-ATP was present in the pipet solution (approximately 70% of initial current remaining after 30 minutes). Inclusion of 5 or 28mM ATP-γ-S, an analog that donates a thiophosphate group resistant to hydrolysis, effectively prevented run-down . The non-hydrolysable analog AMP-PNP was without effect. These results suggest that an ATP-dependent process, possibly phosphorylation, is involved in the functioning of the $GABA_A$ receptor.

PEPTIDES: RECEPTORS IV

394.1

NEUROPEPTIDE Y BINDING TO MEMBRANES FROM BOVINE ADRENAL MEDULLA. T.D. Hexum, C. Cherdchu* and J.D. DEUPREE. Dept. of Pharmacol., Univ. Neb. Med. Ctr., Omaha, NE 68105.

Neuropeptide Y (NPY) can inhibit the nicotinic receptor mediated release of catecholamines from bovine chromaffin cells [Higuchi et al., J. Pharm. Exp. Ther. 244 468 (1988)] suggesting the existence of specific NPY binding sites. Bovine adrenal medulla membranes (P_3 fraction, 0.4 mg/ml) were incubated for 30 min, at 0°, in 0.05 M Tris HCl, pH 7.4, containing 0.005 M $CaCl_2$, 0.005 M $MgCl_2$ and 1 % BSA, in the presence of ^{125}I-NPY. Binding was terminated by rapid centrifugation. Specific binding was determined from the difference between ^{125}I-NPY bound in the presence and absence of 1 x 10^{-7} M unlabeled NPY. Binding was linear over a protein concentration range of 0.1 to 0.8 mg/ml. ^{125}I-NPY was stable for 2 h under these conditions. The binding was saturable and reached equilibrium within 10 min at 0°. Scatchard analysis of specific ^{125}I-NPY binding using the LIGAND computer program indicated a best fit for a two site model with K_D of 2.56 x 10^{-10} and 1.64 x 10^{-7} M and B_{max} of 1.2 x 10^{-11} and 6.0 x 10^{-9} moles/mg protein, respectively. The $t_{1/2}$ for dissociation was 9 min. Displacement curves for NPY-free acid, peptide YY, avian or human pancreatic polypeptide revealed IC_{50}'s greater than 300 x 10^{-9} M for these structurally related compounds. Intact chromaffin cells bind ^{125}I-NPY and have a K_D similar to that found for the high affinity site obtained from P_3 membranes. (Work was supported by American Heart Assoc., Inc.)

394.2

AUTORADIOGRAPHIC DISTRIBUTION OF [^{125}I]PEPTIDE YY BINDING SITES IN RAT BRAIN. J.C. Martel[1], A. Fournier*[2], S. St-Pierre*[2] and R. Quirion[1]. (1) Depts. Psychiat. & Pharmacol. and Douglas Hospital Res. Ctr., McGill University, Montreal, QC and (2) INRS Santé, Pointe-Claire, QC.

Peptide YY (PYY) is a member of the pancreatic polypeptide (PP) family which also includes neuropeptide Y (NPY). Some evidence suggests that there could be different types of PP receptors in the brain (Martel et al., Peptides 9 s1: 15, 1988). Here we report on the distribution of ^{125}I-PYY receptor binding sites in rat brain using an autoradiography technique. Twenty micron thick sections of rat brain were prepared and incubated as described before for ^{125}I-NPY (Martel et al. Peptides 7: 55, 1986) using 25pM ^{125}I-PYY (NEN, 2000 Ci/mmol). Specific binding, defined as the difference in binding occurring in absence and presence of 10^{-6} M cold PYY, accounted for 90 % of binding (septum). Important amounts of ^{125}I-PYY binding sites were found in olfactory bulb, septum, hippocampus, substantia nigra pars compacta and lateralis, some amygdaloid nuclei, cerebellum, area postrema, stria terminalis and fimbria hippocampi. Moderate densities of PYY binding sites were seen in outer layers of the cortex, ventral pallidum, claustrum, striatum, thalamic, hypothalamic and amygdaloid nuclei, ventral tegmental area, central gray, reticular formation and inferior olive. Overall, the autoradiographic distribution of ^{125}I-PYY binding sites resembles that of ^{125}I-Bolton-Hunter NPY binding sites described earlier (Supported by MRCC).

394.3

CHARACTERIZATION OF MULTIPLE NEUROPEPTIDE Y (NPY) RECEPTOR BINDING SITES FROM RAT BRAIN. Mary W. Walker* and Richard J. Miller (SPON: S.P. Grossman) Univ. of Chicago, Chicago, Il 60637

Using mono-iodinated neuropeptide Y (NPY), we observed high, moderate and low affinity receptor populations in rat brain. Only the high and moderate affinity sites were clearly detected in equilibrium studies. The majority of binding was to the moderate affinity population, approximately 50% of which was "lost" in the presence of Gpp(NH)p. In kinetic studies, NPY dissociation was best described by 3 rates. Proportions of intermediate and slow dissociating sites matched proportions of high and moderate affinity sites, respectively, in equilibrium studies. There was also a small proportion of fast dissociating sites. When Gpp(NH)p was added during dissociation, the proportion of fast dissociating sites increased to the same extent that the slow dissociating sites decreased. The proportion of intermediate dissociating sites was unchanged. We propose that rat brain contains a minor population of high affinity binding sites with an intermediate dissociation rate and no sensitivity to Gpp(NH)p. There is also a major population of moderate affinity sites with a slow dissociation rate. A component of these sites can convert to a low affinity state with a fast dissociation rate. Gpp(NH)p enhances conversion by stabilizing the low affinity state, thereby producing a "loss" of moderate affinity binding. Similar patterns have been observed for NPY binding to rat dorsal root ganglion cells, a rich source of NPY receptors. NPY receptors were identified in dorsal root ganglion cells by cross-linking with *Bis*-(sulfosuccinimidyl)suberate after equilibrium binding. We observed a major protein of 39,000 daltons and a minor protein of 55,000 daltons, possibly corresponding to moderate and high affinity binding sites.

394.4

DIFFERENTIAL EFFECTS OF BRADYKININ ANALOGUES ON PHOSPHATIDYLINOSITOL TURNOVER IN JH4 AND N1E-115 CELLS. J.H. Fergus*, R.F. Bruns, and D.T. Dudley*, Parke-Davis Pharmaceutical Research, Ann Arbor, Mi 48105.

Bradykinin (BK) is a nonapeptide that mediates pain, vasodilation and smooth muscle contraction. In both JH4 guinea pig lung carcinoma and N1E-115 mouse neuroblastoma cells labeled to equilibrium with [^{3}H]inositol and stimulated with 100 nM BK there is a rapid and large increase in inositol trisphosphate followed by increases in inositol tetrakis-, bis- and monophosphates. These increases peak at 2 min and return to basal levels by 10 min. No effect of stimulation is seen on inositol pentakis- and hexakisphosphates. For routine studies, [^{3}H]inositol phosphate (IP) accumulation in the presence of Li^+ was monitored. Both JH4 and N1E-115 cells have comparable EC_{50} values for BK (8.2 *vs* 12.8 nM); however, JH4 cells consistently show greater stimulation (10 fold *vs* 5 fold). The selective B_1 subtype receptor agonist (des-Arg^9)-BK is inactive at 100 μM in both cell lines, suggesting the [^{3}H]IP accumulation is mediated by B_2 subtype receptors. However, the rank order of potency of a variety of BK analogues is quite different between the two cell lines. Additionally, (D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7)-BK (NPC-349), which is known to act as a potent, competitive BK antagonist in several smooth muscle systems, is a competitive BK antagonist in JH4 cells, yet acts as a full agonist in N1E-115 cells. These results identify JH4 cells as a sensitive and useful model for studies of the signal transduction and pharmacology of BK receptors and suggest that significant heterogeneity may exist among B_2 subtype receptors.

394.5

CHARACTERIZATION OF ^{3}H-BRADYKININ BINDING SITES IN GUINEA-PIG CNS AND NASAL TURBINATE. Y.Fujiwara*, C.R.Mantione[1] and H.I.Yamamura. Univ. of Arizona, Coll. of Med., Tucson, AZ 85724. [1]Procter & Gamble Company,Cincinnati, OH 45247.

Bradykinin(BK) acts as a putative neurotransmitter and as a mediator of inflammatory reactions. We have obtained evidence for specific high affinity BK binding sites in guinea-pig whole brain(WB), spinal cord(SC) and nasal turbinate(NT). These tissues of adult male guinea-pigs were homogenized in 25mM TES buffer and centrifuged twice. Membrane preparations were incubated with 0.1nM ^{3}H-BK in 1.0 ml of assay buffer (25mM TES, 1mM 1,10-phenanthroline, 140ug/ml bacitracin, 2uM captopril, 1mM DTT and 0.1% BSA) at 25°C for 100 min. Specific binding was determined in the presence or absence of 1uM unlabeled BK.

Specific bindings in WB, SC and NT were linear between 0.2~2.0% tissue weight and reached equilibrium within 30 min at 25° C in the kinetic experiments. ^{3}H-BK binding (<1.0 nM) was saturable and indicated the presence of a high affinity site in these tissues. In competition experiments, D-Phe^7-BK(a B_2 antagonist) inhibited ^{3}H-BK binding (IC_{50}=60nM) while des-Arg^9(Leu^8)-BK(a B_1 antagonist) had no effect at 1.0 uM. These studies indicate the presence of B_2 BK receptors in the guinea-pig WB, SC and NT.

394.6

BINDING OF THE GROWTH HORMONE RELEASING PEPTIDE SK&F110679 TO SPECIFIC HYPOTHALAMIC AND PITUITARY BINDING SITES. E.E.Codd and R.F.Walker* Smithkline and French Labs., P.O. Box 1539, King of Prussia, PA 19406

SK&F 110679 (His-D-Trp-Ala-Trp-D-Phe-LysNH_2)is an enkephalin-derived hexapeptide which specifically releases growth hormone in a wide variety of species in vivo and in vitro. Previous binding studies, using ligands specific for mu and delta opioid binding sites, demonstrated an inverse relationship between the opioid binding potency and the growth hormone releasing effectiveness of a series of SK&F 110679-related peptides (Codd and Walker, Neuropharm., 1988). In an attempt to better understand its mode of action, we established a binding assay for the peptide using a ligand which had been tritium labeled at the histidine residue. Membrane fragments from both hypothalamic and anterior pituitary tissue were found to contain sites to which ^{3}H-SK&F 110679 reversibly and saturably bound. The binding curves for ^{3}H-SK&F 110679 to both hypothalamus and anterior pituitary membrane fragments were resolved into two binding components with the computer program LIGAND. The K_d's obtained were in the 10^{-9}M and 10^{-8}M range. The relation of these binding sites to the peptide's growth hormone releasing activity was explored by examining the relationship between the binding potency and growth hormone releasing effectiveness of a series of peptides related to SK&F 110679. For both hypothalamic and pituitary sites, a significant correlaton between binding and growth hormone release was obtained. Thus these binding sites appear to be involved in the release of growth hormone by SK&F 110679 related peptides.

394.7

DIFFERENT DISTRIBUTIONS OF EPIDERMAL GROWTH FACTOR-LIKE IMMUNOREACTIVITY (EGF-LI) AND IMMUNO REACTIVE EGF-RECEPTOR (IR-EGF-R) IN RAT SPINAL CORD NEURONS. E.V. Joshy,* V. Askanas, W.K. Engel, USC Neuromuscular Center, Los Angeles, CA 90017.

Distribution patterns of EGF-LI and IR-EGF-R in adult rat (Sprague-Dawley) spinal cord sections were compared using peroxidase-antiperoxidase immunocytochemical localization.

EGF-LI was observed in the cytoplasm of neurons of ventral, intermediate, and dorsal horns without apparent difference of staining intensity. Nuclei of some neurons also showed EGF-LI. The distribution of IR-EGF-R was distinctly different. There was no IR in the small dorsal-horn neurons. In the ventral horn there was intense cytoplasmic staining of the larger neurons (>20 um) and lesser staining of the smaller neurons (<20 um) in lamina 7.

Speculatively, EGF might be a transported-exported trophic peptide interacting with post-synaptic EGF receptors in the spinal cord. As suggested by the differential distribution of EGF and its receptors in this study, EGF synthesized in sensory neurons of the dorsal-horn may interact with EGF receptors on large motor neurons. Thus, failure of endogenous spinal EGF, could affect motor more than sensory neurons, and this might be relevant to diseases of preferential motor neuron degeneration.

394.8

A STUDY OF THE SPECIFIC BINDING IN RAT BRAIN MEMBRANES AT THE GASTRIN RELEASING PEPTIDE RECEPTOR AND THE SECOND MESSENGER SYSTEM ASSOCIATED WITH THIS BOMBESIN-LIKE PEPTIDE RECEPTOR. E.B. Hollingsworth. Dept. of Pharmacology, Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709.

Gastrin releasing peptide, (GRP) binds specifically to rat brain membranes. The binding is saturable, dependent on the tissue concentration, time of incubation and the pH of the buffer. Saturation curves revealed a high affinity site with a Kd equal to 2.4 nM and a B_{max} equal to 6 pmoles/g wet weight of tissue. A lower affinity site is indicated but is not believed to be relevant. Regional distribution studies revealed that the hippocampus contained the highest concentration of binding sites. Hippocampal membranes contained almost twice as much as the tissue with the next highest concentration. The cerebellum contained the lowest density of sites. The affinity of GRP and bombesin and their analogues for the GRP receptor was determined. GRP (14-27) and [Tyr^4] bombesin had the greatest affinity for the receptor of the peptides tested. GRP (1-16) had no affinity for the receptor indicating that the carboxyl end of the peptide is needed for recognition of the peptide for the receptor. Bombesin was shown to stimulate the breakdown of phosphatidylinositol in rat brain hippocampal minces. The concentration required to produce a half maximal effect was in the namomolar range which corresponds to the affinity of bombesin for the high affinity site on the GRP receptor.

394.9

ANTAGONISM OF CENTRAL BOMBESIN (BN) RECEPTORS BY (Psi^{13,14}, Leu^{14})BN. Z. Merali[1], T.W. Moody[2] and D.H. Coy[3]. [1]Psychology & Pharmacology, Univ. of Ottawa, Ontario, K1N 9A9. [2]Biochemistry, George Washington Univ. Washington D.C. 20037. [3]Dept. of Medicine, Tulane Univ. New Orleans, LA 70112.

This study assessed the ability of (Psi^{13,14}, Leu^{14})BN to serve as a central BN antagonist. The in vitro receptor binding studies on fresh frozen coronal rat brain slices, revealed that (Psi^{13,14} Leu^{14})BN inhibited specific (^{125}I-Tyr^4)BN binding activity with IC_{50} value of 100 nM. In contrast, (Psi^{9,10}, Leu^{14})BN and (Psi^{12,13}, Leu^{14})BN were less potent with IC_{50} values of 1000 and 3000 nM respectively. The in vivo studies were done on rats on a regimen of 17.5 hr of food deprivation, followed by i.c.v administration of (Psi^{13,14}, Leu^{14})BN (0.1-10 ug) and/or BN (0.5 ug), meal presentation, and behavioural monitoring. BN decreased the meal size by about 60% and increased grooming by over 5-fold. (Psi^{13,14}, Leu^{14})BN antagonized both these effects, starting at a dose of 0.5 ug. At the dose of 10 ug, it completely blocked the effect of BN on food intake but only partially blocked its effects on grooming. The antagonist alone failed to alter behavior. Thus this pseudo peptide may serve as a pure BN antagonist in the rat CNS, with slightly better in vivo potency than the earlier compounds.

394.10

CHANGES IN TRH RECEPTORS IN THE BRAIN OF HIBERNATING AND EUTHERMIC GROUND SQUIRRELS. S.B. Caine*, T.L. Stanton and A. Winokur. Dept. of Pharmacology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104 and the Dept. of Physiology, CSU, Long Beach, CA 90840.

Thyrotropin-releasing hormone (TRH) exerts state-dependent physiological and behavioral effects when microinjected into the dorsal hippocampus of ground squirrels (Citellus lateralis). To examine the neurochemical correlates of these changes, we used quantitative autoradiography to localize and quantify TRH receptors in the CNS of hibernating (H), winter euthermic (WE) and summer euthermic (SE) animals (n=4/grp). The pharmacological profile of [^{3}H]-MeTRH binding to brain sections was similar to that in the rat with a Kd of 6.7 ± 0.1 nM. Analysis of 82 discrete regions of the brain revealed statistically significant differences for only eight areas ($p<0.05$-0.001). Seasonal changes (SE vs WE) were reflected by decreases in TRH receptor binding in the arcuate nucleus, dorsomedial nucleus, and the ventral pallidum of WE animals. Increased binding in WE animals was evident in the area surrounding the suprachiasmatic nucleus. State dependent changes (WE vs H) were characterized by decreases in TRH receptor binding in the paraventricular nucleus, medial preoptic area and the ventral tegmental area of H animals. Increased binding occurred in the anterior cortical nucleus of the amygdala in H animals. These findings indicate that seasonal and state dependent changes occur in TRH systems in the brain, and further support the hypothesis that naturally occurring changes in central TRH systems may mediate the level of arousal in this species.

394.11

CALIBRATION OF AMERSHAM HYPERFILM ß-MAX™ (HFBM) WITH [14C] STANDARDS FOR QUANTITATIVE AUTORADIOGRAPHY (QAR) WITH [125I]. Denis G. Baskin and Thomas H. Wimpy* (SPON: James W. Little). Departments of Medicine and Biological Structure, University of Washington, Seattle, WA 98195, and V. A. Medical Center, Seattle, WA 98108.

HFBM produces high contrast autoradiographic images with [125I]. In order to use HFBM for QAR, we calibrated the response of this film to [14C] plastic standards in terms of tissue-equivalent concentrations of [125I]. Plastic sections with standard concentrations of [125I] (Amersham "Microscales") and [14C] (American Radiolabeled Chemicals) were apposed to HFBM for 1, 3, 5, and 9 days, along with liver and muscle slices (20 um) labeled with increasing concentrations of [125I]-insulin. Optical densities produced on HFBM by [125I] and [14C] plastic standards were converted to equivalent tissue [125I] concentrations (DPM/mm2). The responses of HFBM to the plastic [125I] and [14C] standards (each calibrated in tissue-equivalent DPM/mm2) were similar ($p<.001$). Standard curves of tissue [125I] (DPM/mm2) vs. standard [14C] (uCi/g) concentrations fit second order polynomials ($r2 = .99$-1.0; $p<.001$): 1 day, $y = -26.8+1.0x-.00013x2$; 3 day, $y = -49.3+1.4x-.001x2$; 5 day, $y = -39.4+1.6x-.001x2$; 9 day, $y = -5.6+1.5x-.002x2$. The useful DPM/mm2 ranges for measuring tissue [125I] radioactivity with the [14C] plastic standards were: 1 day, 50-1450; 3 day, 25-675; 5 day, 10-400; 9 day, 5-210. The results indicate that [14C] plastic sections are valid standards for measuring [125I] radioactivity concentrations in tissue slices by QAR with Hyperfilm ß-Max™. (Supported by NIH grant NS24809 and the Veterans Administration)

394.12

GLIA IN THE CIRCUMVENTRICULAR ORGANS: A POSSIBLE ROLE IN PROTECTING THE BRAIN FROM BLOOD-BORNE PEPTIDES. M. Ian Phillips, Tammy Vittands* and Rod Casto. Department of Physiology, College of Medicine, University of Florida, Gainesville, FL. 32610.

The circumventricular organs (CVOs) have been called "windows on the brain" because their capillaries lack the tight junctions which are necessary for a blood-brain-barrier (BBB). However, such windows are not open to free flow of normally excludable proteins and peptides. Otherwise, the BBB would constantly be circumvented. Therefore, a mechanism must exist, specific to the CVOs, which prevents uncontrolled diffusion of plasma peptides into brain. We propose that glial cells effect this mechanism.

To demonstrate the presence of glia in CVOs, rats were perfused and brain sections immunocytochemically stained using GFAP antibodies for astroglia. There are numerous glia inside the body of the organ (SFO and OVLT). In the area postrema (AP) there is a border of glial cells surrounding the organ and separating it from the NTS and other brain structures. Dye injections into the AP did not flow out of the border and dye in NTS did not flow into the AP. Glia cells contain angiotensin II receptors (Raizada, Phillips et al., PNAS, 1987) and the CVOs have high affinity binding sites for Ang II. Therefore, it is proposed that CVO glia rapidly bind and metabolize blood-borne peptides arriving at CVOs. This mechanism restricts normal plasma concentrations of peptides to the CVO and prevents crossing into brain tissue. (Supported by NIH 1-R01-27334 to MIP.)

394.13

IN-SITU DEMONSTRATION THAT SUBSTANCE P RECEPTORS ARE EXPRESSED BY GLIA AFTER NEURONAL INJURY. R.P. Zimmerman, T.S. Gates*, C.G. Boehmer*, D.J. Johnson and P.W. Mantyh. Center for Ulcer Research and Education; Brain Research Institute; UCLA School of Medicine, Los Angeles, CA 90024

Previous studies have shown that glia can express receptors for various neurotransmitters in-vitro. In this report, we use quantitative receptor autoradiography to determine in-situ which receptor binding sites are expressed by the pure glial scar formed following transection of the optic nerve in the albino rabbit. After unilateral optic nerve transection, the rabbits were allowed to survive for 40-99 days. The brains were removed, embedded in Tissue-Tek, frozen to -70°C, and sectioned (30 µm). The sections were then processed for quantitative receptor autoradiography with either radiolabeled bombesin, calcitonin gene related peptide, cholecystokinin, galanin, glutamate, somatostatin, substance P (SP) and vasoactive intestinal peptide.

Specific binding sites were identified in the forebrain for each ligand, but no specific binding sites for any radioligand tested were expressed in the normal optic nerve or tract. In the lesioned optic nerve and tract, SP was the only radioligand of those tested for which specific receptor binding sites were observed in the glial scar tissue. This glial scar expresses one of the highest concentration of SP binding sites seen in the rabbit brain, and pharmacological experiments show that these binding sites have a similar pharmacological profile to SP receptors in the striatum.

This study demonstrates that presumed glia populating the scar formed after transection of the optic nerve ectopically express high concentrations of SP receptors and suggest that SP may play a role in regulating the glial response to neuronal injury.

394.14

EFFECTS OF INTRATHECAL NEUROKININ RECEPTOR AGONISTS ON THE CARDIOVASCULAR SYSTEM OF THE CONSCIOUS FREELY MOVING RAT. H. Hasséssian and R. Couture*. Dept. of Physiology, Faculty of Medicine, University of Montréal, Montréal, Qué., Canada.

This study aims to characterize the neurokinin (NK) receptor which mediates the cardiovascular responses elicited by the intrathecal (i.th.) administration of SP. In addition to SP, neurokinin A (NKA) and neurokinin B (NKB), five NK receptor subtype selective agonists: [Pro^9, $Met(O_2)^{11}$]SP, (A); [ß-Ala^4, Sar^9, $Met(O_2)^{11}$]SP (4-11), (B); [Nle^{10}]NKA (4-10) (C); [ß-Asp^4, $MePhe^7$]NKB (4-10), (D) and [Succinyl-Asp^6, $MePhe^8$]SP (6-11), (E) were studied for their effects on mean arterial pressure (MAP) and heart rate (HR). Male Wistar rats were cannulated under pentobarbital anesthesia, with two catheters: a PE-10 in the intrathecal space (T_8-T_{10} vertebral level) and a PE-50 in the caudal artery. Experiments were performed 24h later in the conscious freely moving rat. The three NK elicited a pressive effect with the following rank order of potency: SP > NKA = NKB. Only the response to SP was dose dependent (0.065-65 nmol). All NK elicited a dose dependent increase in HR with the following rank order of potency: SP > NKA > NKB. The NK-1 selective agonists (A and B) produced a maximal increase in MAP and HR greater in intensity than SP and the HR response they elicited lasted longer. The NK-2 (C) and NK-3 (D and E) selective agonists elicited MAP increases comparable to SP but produced a much weaker HR response. In conclusion, the cardiac and pressive responses elicited by the intrathecal administration of SP appear to be mediated by an NK-1 receptor subtype. (Supported by the MRC of Canada).

394.15

SYMPATHETIC NEURONS EXPRESS HIGH LEVELS OF RECEPTORS FOR SENSORY NEUROPEPTIDES T.S. Gates*, R.P. Zimmerman, C.G. Boehmer*, S.R. Vigna, J.E. Maggio, and P.W. Mantyh. Brain Res. Inst., UCLA, LA, CA 90024, Dept. Biol. Chem. and Mol. Pharm., Harvard Med. School, 02115, Dept. of Phys., Duke Univ. Med. Center, 27710

To define which sensory neurotransmitters are involved in regulating post-ganglionic sympathetic neurons we used quantitative receptor autoradiography to determine which neuropeptide receptor binding sites are expressed by neurons in the rat and rabbit superior cervical ganglion and rabbit superior mesenteric ganglion. The neuropeptides examined included bombesin, calcitonin gene related peptide, cholecystokinin, galanin, somatostatin, substance K, substance P and vasoactive intestinal peptide. High levels of receptor binding sites for cholecystokinin, galanin, somatostatin, substance P and vasoactive intestinal peptide were present in all sympathetic ganglion examined whereas specific receptor binding sites for bombesin, calcitonin gene related peptide and substance K were not detectable. These results suggest that sensory neurons use specific neuropeptides to modulate sympathetic activity.

394.16

FUNCTIONAL ROLE OF HIGH AFFINITY NEUROTENSIN (NT) BINDING SITES IN RAT BRAIN. S. Bischoff, M. Heinrich*, E. Küng*, M. F. Pozza*, M. Schaub*, W. Schilling* and A. Vassout*. Pharma. Research, CIBA-GEIGY, Basel, CH-4002 Switzerland.

High and low affinity NT binding sites were characterized with (^{125}I-Tyr^3)NT and (^{3}H)NT binding assays. (^{3}H)NT binding was displaced by 80% with levocabastine (L), a marker of low affinity NT sites. NT and a synthetic pentapeptidic analog (P) had IC_{50}'s of 21 and 39000 nM. In (^{125}I)NT binding, L exerted a maximum of 50% inhibition, in contrast to NT (IC_{50}: 2.3 nM) and P (IC_{50}: 175nM). P showed a Hill coef. of 0.6, indicating a clear-cut interaction with two sites; the appropriate curve fitting revealed IC_{50}'s of 13 and 683 nM. These data confirm that (^{125}I)NT binds to high and low affinity NT sites. They show also that L is specific for the low, and P strongly selective for the high affinity NT sites. In vitro, P activated nigral dopamine neurons (in slices) at 3 nM (maximum: 0.3 µM). L also tended to excite these neurons, but the shape was atypic, the concentration very high (3 µM) and the solution unphysiologic (DMSO). In vivo, P produced a dose-dependent hypothermia after icv injection (first effect at 0.1 µg/rat), whereas L was inactive at the highest usable dose (70 µg/rat). In conclusion, P mimicked in vitro and in vivo effects of NT, being 3-5 times more potent, whereas L was rather inactive. According to the selectivity of P for high affinity NT binding sites, these sites play a functional role.

394.17

THE CALCITONIN RECEPTOR. R.C. Dana. Dept. of Medicine, Univ. of Calif., San Diego, La Jolla, CA 92093.

Specific, high-affinity (Kd<1 nM) cell membrane receptors for calcitonin and calcitonin-gene related peptideare widely distributed throughout the central and peripheral nervous systems and also many somatic tissues. Radioiodinated salmon calcitonin was used to study the binding to receptors on isolated membranes from: neonatal rat brain, adult rat brain, kidne liver, 8 different cell lines, bovine kidney, human placenta. The highest levels of specific binding per mg of protein were found in brains from one week rat pups. Trichloroacetic acid precipitation revealed that the liver had the greatest amount of degredation activity of peptide. Cross-linking studies revealed the neonatalrat brain receptor tohave a molecular weight of 60-70K. Current research involves the purification of receptor by affinity chromatography and electrophoresis, partial amino acid sequence analysis for the eventual isolation of the receptor cDNA with oligonucs..

394.18

MULTIPLE AFFINITY STATES OF BINDING SITES FOR (+)SKF10,047 AND 1-[1-(2-THIENYL) CYCLOXYL]-PIPERDINE (TCP) IN RAT AND GUINEA PIG BRAIN MEMBRANES. G-Z.Zhou*, A.Katki*, P.Munson* & D.Rodbard* (SPON:B.Cox),LTPB,NICHD, NIH, Bethesda,MD 20892.

We employ program "LIGAND" for simultaneous analysis of self- and cross- displacement studies and of "multiligand" dose-response surfaces, with or without 10-50 nM haloperidol (HAL), (48 curves or 574 points) to characterize multiple subtypes of binding sites for (+)SKF10,047, phencyclidine (PCP), and its analogs (TCP and 3-OH-PCP) in membranes from rat and guinea pig brain. We find 3 distinct sites in rat brain ($p < 0.001$): one (+)SKF10,047 selective, HAL suppressible site and 2 TCP selective, HAL nonsuppressible sites (Table). In guinea pig, we find 4 classes of sites: 2 selective for (+)SKF10,047 (only one is suppressible with 10-50 nM HAL) and 2 are TCP selective (Table):

		Binding Capacity	Dissociation Constants, Kd (nM)	
	site	(pmol/mg prot.)	(+)[^{3}H]SKF10,047	[^{3}H]TCP
Rat	1	1.0 ± 0.3	100 ± 25	8.6 ± 1.9
	2	2.8 ± 0.4	>1000	72 ± 20
	3	0.5 ± 0.2	120 ± 47*	>1000
Guinea pig	1	1.3 ± 0.1	330 ± 29	7.4 ± 0.6
	2	2.1 ± 0.7	>10000	310 ± 100
	3	4.3 ± 0.9	620 ±140	>1000
	4	0.4 ± 0.1	12 ±2.4	95 ± 16

*suppressible with 10-50 nM HAL. The rank order of potency for 12 drugs was evaluated for the predominant sites. The apparent multiciplicity of sites may help to clarify function, localization and modulation of these receptors.

394.19

QUANTITATIVE AUTORADIOGRAPHY OF INSULIN-LIKE GROWTH FACTOR -I (IGF-I) RECEPTORS IN RAT BRAIN: THE EFFECT OF FIXATION. M.G. King*, T.H. Wimpy*, and D.G. Baskin. Depts. Biological Structure and Medicine, University of Washington, Seattle, WA 98195, and V.A. Med. Center, Seattle, WA 98108.

We evaluated the effect of paraformaldehyde (PAF) on the binding characteristics of brain IGF-I receptors in order to develop a method for localizing IGF-I receptors at the cellular level. Frozen sections (20 µm) from rat brains perfused with saline (control), 1% PAF, or 2% PAF were incubated in 0.05 nM ^{125}I-IGF-I for 20 h at 5°C, with or without unlabeled IGF-I (0.25-50 nM) or related peptides (IGF-II and insulin), and apposed to Hyperfilm ß-Max (Amersham) for 3 days. The binding of IGF-I to 10 brain regions (including median eminence, CA3, and cerebral cortex) was quantified by computer densitometry of the autoradiographic images. The 3 groups (control, 1% PAF, 2% PAF) were not significantly different for specific binding (79 ± 1.0%, 76 ± 1.0%, 82 ± 1.0%), binding specificity (IGF-I>IGF-II>insulin), and the reversibility of binding. The number (B_{max}) and affinity (K_d) of IGF-I receptors of unfixed brain (2.03 ± 0.2 pmol/mm^2, 2.41 ± 0.2 nM) was not affected by 1% PAF (1.78 ± 0.2 pmol/mm^2, 2.46 ± 0.2 nM). 2% PAF increased the B_{max} (3.71 ± 0.5 pmol/mm^2) and decreased the K_d (4.59 ± 0.5 nM), but total specific binding capacity was unchanged. These findings indicate that tissue fixed in PAF is suitable for localization and characterization of IGF-I receptors. (Supported by NIH Grant NS 24809 and the V.A.).

PEPTIDES: ANATOMICAL LOCALIZATION III

395.1

ENKAPHALINERGIC INPUT TO THE ANTERIOR THALAMUS. B.R. Rittberg*, P.L. Faris*, J.J. Sikora*, S.A. May*, and B.K. Hartman. (SPON: M. Dysken) Dept. of Psychiatry, Univ. of Minnesota, Minneapolis, MN 55455.

The rat thalamus contains a dense plexus of met-enkephalin containing nerve fibers and terminals. The cell bodies that give rise to this plexus have not been identified. This study proposes a source for this innervation based on retrograde transport and immunohistochemistry.

Small discrete injections of the retrograde tracer Fluro Gold (20nl of a 4% solution) were centered in the antero-ventral nucleus of the thalamus in adult Wistar rats. Five days later, the rats were sacrificed and serial brain sections were studied with florescence microscopy. Numerous known thalamic afferents had been labeled. These labeled nuclear groups were then compared to enkephalin-positive cells of other rats to determine overlap. This second group of rats had been pre-treated with intraventricular colchine (100 ug). Forty-eight hours later the animals were perfused with 2% parformaldehyde and the brains serially sectioned (40 micron). The sections were then incubated with anti-methionine enkephalin which was visualized either by the PAP method or immunofluoresence. Analysis of overlap indicated that the intrinsic thalamic neurons, the thalamic reticular nucleus, caudal bed nucleus of the stria terminalis and zona incerta were the most likely sources of the thalamic inputs. Verification of this hypothesis will require the simultaneous application of these two techniques. The delineation of the enkephalinergic inputs to thalamus may be an aid to the understanding of central nociceptive pathways. Supported by NS-12311 (BKH), RSDA MH-00595 (PLF).

395.2

QUALITY OF IMMUNOHISTOCHEMISTRY AS A FUNCTION OF CONCENTRATION OF REAGENTS AND INCUBATION PARAMETERS. J.J. Sikora*, P.L. Faris* B.R. Rittberg* B. Moore[1], and B.K. Hartman. (SPON: P.Santi) Dept. of Psychiatry, Univ. of Minn., Mpls., MN 55415, [1]Wash. U. Sch. Med., St. Louis, MO 63110.

Published immunohistochemical procedures vary widely in times of incubation/washes and the concentration of immunoreagents. While some differences might be expected for the primary antibodies, an optimal set of conditions should exist for the secondary detection systems. We examined the application of the PAP localization system on rat brain 40µm vibratome sections fixed by perfusion with pH 8.5 2% formaldehyde/0.05% glutaraldehyde. Localization of the glial protein S-100 was chosen as the prototype antigen because it is present in many cellular elements of varying size throughout the thickness of the section. The antiserum used was sufficiently high in titer and affinity to permit use at a 1/20,000 dilution, making it's contribution to nonspecific staining negligible. Thus S-100 is nearly an ideal marker for evaluating the quality of staining.

Concentrations of anti-rabbit IgG and PAP were varied from 1/50 to 1/5000 of the undiluted reagents and with incubation times ranging from 2h. to 72h. Excess concentrations of either linking antibody or PAP, result in marked loss of resolution in fine structures with only a modest increase in intensity. Optimal times for clear visualization of structures throughout the section were between 18 and 24 hrs. At high concentrations of immunoreagents, staining was achieved throughout the section in a shorter time (1 to 4 h.), but localization in the outer layers of the section were overstained while structures within the section were understained. No conditions could be found where short incubations approached the quality of long incubations with low reagent concentrations. These results apply to the visualization of enzymes, peptides, and other protein markers. NS-12311 (BKH), RSDA MH-00595 (PLF)

395.3

SIMULTANEOUS LOCALIZATION OF CCK AND MET-ENKEPHALIN (ENK) IN THE RAT HYPOTHALAMUS. J.P. Kettinger*, P.L. Faris*, B. Rittberg, B.K. Hartman, J. Chen, A. Nadzan and J.F. McKelvy. Departments of Psychiatry and Pathology, Univ. of Minnesota, Mpls, MN 55455, and Abbott Laboratories, Abbott Park, IL 60064.

CCK and ENK co-exist in discrete CNS regions of the rat, including several thalamic nuclei and specific cortical layers (Gall et al., Brain Research 403: 403, 1987). In light of evidence from our lab and others (e.g. Itoh et al., Eur. J. Pharm., 80: 421, 1982) that CCK may function as a physiological antagonist of opioid action, the finding that CCK and ENK are co-stored and possibly co-released suggests the antagonistic relationship between these peptides may be mediated at the post-synaptic receptor complex. However, reported incidences of co-existance is minimal at hypothalamic sites where CCK and ENK exert opposite effects on food intake. We sought to identify other cell-to-cell interactions that may underlie their behavioral effects.

Formaldehyde fixed sections were double-labeled using a mouse monoclonal antibody (AB) against CCK-8 and a rabbit polyclonal AB against ENK followed by incubation with anti-mouse (goat)-FITC and anti-rabbit (goat)-Texas Red. Mis-matching the secondary antibodies resulted in total abolition of immunostaining.

Accessory magnocellular cells were immunoreactive for CCK and ENK-containing fibers surrounded these nucleur groups with little penetration to deeper lying cells. Approximately 5% of immunolabeled ENK cells in the parvocellular periventricular region also contained CCK. The dorsal parvocellular division was innervated with fibers containing both peptides, suggesting modulation of a common post-synaptic site. These findings suggest that CCK may interact with ENK by multiple mechanisms. Supported by NS-12311 (BKH), RSDA MH-00595 (PLF).

395.4

DISTRIBUTION OF GASTRIN-RELEASING PEPTIDE (GRP) mRNA-CONTAINING CELLS IN RAT BRAIN AS SHOWN BY *IN SITU* HYBRIDIZATION. R.T.Zoeller, A.-M. Lebacq-Verheyden* and J.F. Battey*. Lab of Neurochemistry, NINCDS, NIH, Bethesda, MD 20892.

GRP is a 27 amino acid peptide representing the mammalian homolog to the amphibian peptide bombesin. Although this peptide was initially identified as a gut hormone, central effects of GRP have been described, including effects on appetite, glucose metabolism, growth hormone and prolactin secretion. Radioimmunoassay and immunocytochemical evidence support the view that GRP is synthesized in the brain. However, antisera against GRP may cross-react with a variety of related peptides. Recently, Lebacq-Verheyden et al. (*Molecular Endocrinology,* In Press) isolated and sequenced cDNA clones encoding GRP from a rat brain library, thereby demonstrating that preproGRP mRNA is synthesized in rat brain. The present study was undertaken to map the distribution of GRP mRNA-containing cells in the rat brain. Brains from male and female rats were fresh-frozen and sectioned at 12μm in a cryostat in either coronal or saggital plane. We used NTB-3 emulsion and both ^{35}S-cRNAs (tested for specificity by Northern analysis) and synthetic oligomers to visualize GRP mRNA-containing cells. Labelled cells were distributed widely throughout the brain, especially cingulate cortex, mPOA, SCN, amygdala, and dentate gyrus. We failed to detect GRP mRNA in areas such as PVN and ventral SCN which consistently exhibit bombesin/GRP-like immunostaining. These results further support a role for GRP in central function, and clarify inconsistencies in the literature relating to its distribution within the brain.

395.5

POSTNATAL CHANGES IN THE DENSITY AND DISTRIBUTION OF NEUROTENSIN-IMMUNOREACTIVE FIBERS IN THE MEDIODORSAL THALAMIC NUCLEUS OF THE RAT. J. P. Ray and J. L. Price, Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

A previous report (Inagaki et al., Brain Res., 260, 143-146, '83) has suggested that the peptide neurotensin is contained in neurons of the piriform cortex which project to the mediodorsal thalamic nucleus (MD) in young rats. To confirm this, we have studied the distribution of neurotensin-immunoreactive (NTIR) fibers in MD using three antisera directed at different parts of the neurotensin molecule (Emson et al., J. Neurochem., 38, 992-999, '82). In adult rats, NTIR fibers in MD are sparse, located mostly at the medial edge of MD with a few, poorly-stained NTIR fibers in the central part of MD. In contrast, during the first postnatal week, both the medial and central portions of MD stain heavily for neurotensin. The density of NTIR fibers in MD then progressively decreases until the density typical of adult rats is reached, at about 5 weeks. The number of NTIR neurons in the piriform cortex declines approximately eightfold during this period. Changes in the distribution of NTIR fibers in MD also occur. In seven day old rats, the medial and central patches of NTIR fibers are contiguous, but by ten days a non-immunoreactive zone forms between them. This non-immunoreactive zone enlarges until the medial contingent of NTIR fibers reaches its adult position at the medial edge of MD.
Supported by NIH research grant NS09518.

395.6

A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF GABA IMMUNOSTAINING IN MONKEY SPINAL CORD. S.M. Carlton, Anat. & Neurosci. Dept., U. Texas Med. Br., Galveston, TX 77550.

The transmitter γ-aminobutyric acid (GABA) has been previously localized in rat at the light (LM) and electron microscopic (EM) levels. These studies suggest a widespread distribution of GABA neurons and terminals in the spinal cord dorsal and ventral horns. In the present study, GABA-containing profiles were immunostained and mapped at the LM level in the monkey lumbar spinal cord. EM analysis of GABAergic neurons and terminal interactions are also reported. Four animals (M. fascicularis) were perfused with mixed aldehydes and the lumbar cord removed. The tissue was sectioned at 25μm on a vibratome and immunostained with anti-GABA using the PAP technique. LM analysis demonstrated GABAergic cells concentrated in the superficial dorsal horn (DH); however, they were present in all spinal laminae except lamina IX. Small diameter oval to round cells were located in the upper laminae while larger diameter cells with visible dendrites were present in deeper layers. Punctate GABAergic profiles, presumably terminals, were scattered diffusely over the OH, lamina X and around motoneurons in the ventral horn. EM analysis demonstrated both myelinated and unmyelinated GABAergic axons. GABAergic cell bodies with large immunoreactive nuclei were seen. GABAergic terminals contained mainly round clear vesicles and often a few dense core vesicles and synapsed onto labeled and unlabeled somata, dendrites and other axon terminals. (Supported by NS11255.)

395.7

RETINAL TERMINALS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT ARE IMMUNOREACTIVE FOR N-ACETYLASPARTYLGLUTAMATE. S.B. Tieman, Neurobiol. Res. Ctr., SUNY Albany, NY 12222.

We have previously identified N-acetylaspartylglutamate immunoreactivity (NAAG-IR) in the retinal ganglion cells (RGC) of the cat and in the neuropil of their targets. If NAAG serves as a transmitter for RGC, as we have suggested, then it should be found in their terminals. To determine whether these terminals contain NAAG, one adult cat was deeply anesthetized and one eye removed. The cat was reanesthetized 10 days later and perfused with PBS followed by 4% paraformaldehyde and 4% 1 ethyl 3-(3-dimethylaminopropyl) carbodiimide in 0.1 M phosphate buffer. Frozen sections of LGN were processed for NAAG-IR by the ABC method. NAAG-IR was reduced in the neuropil, but not the cell bodies, of the denervated layers, suggesting that this loss of IR was due to the degeneration of the retinal terminals. Further, an unoperated adult cat was perfused as above, and vibratome sections of LGN were processed by the PAP method, postfixed in osmium tetroxide, and processed for electron microscopy. There was label within large terminals containing round vesicles and mitochondria that were less dense than those in other profiles, and making asymmetric synapses onto dendrites. These results demonstrate that retinal terminals are immunoreactive for NAAG, which is consistent with a neurotransmitter role for this peptide.

395.8

CA2 (RESISTANT) SECTOR OF HUMAN HIPPOCAMPUS DEFINED BY CHROMOGRANIN-LIKE IMMUNOREACTIVITY. D.G. Munoz, D.H. George* and L. Kobylinski*. Dept. of Pathology, Univ. of Saskatchewan, Saskatoon, Saskatchewan, Canada. S7N 0W0

Status epilepticus and cerebral anoxia can result in the destruction of most neurons in the hippocampus, but characteristically spare a narrow sector, known as the resistant sector, that approximately correspond to the CA2 sector defined by Golgi stains. Although certain enzymes show distribution gradients among the hippocampal sectors, there has been no reports of specific CA2 markers. Here we show that such sector can be sharply defined by the perikaryal chromogranin-like immunoreactivity of its pyramydal neurons. The monoclonal antibody (LK2H10) produced against a human pheochromocytoma (Wilson BS & Lloyd RV, AM J Path 115: 458, 1984) recognizes the human chromogranin A molecule as well as an array of polypeptides (chromogranins) with MW down to 28,000. Vibratome sections of 6 normal human hippocampi fixed for 24 hours in PPG fixative (Neuroscience 7:1779-1783, 1982) were immunocytochemically stained with LK2H10 by the ABC method. In all cases there was intense staining of the mossy fibers, as well as granular cytoplasmic staining of all pyramidal neurons in a sector corresponding to CA2, stretching from the termination of the mossy fibers to a sharp wedge-shaped lateral limit. No other neurons in the hippocampus proper were stained, although there was weak immunostaining of a subset of neurons in the dentate gyrus and the hilum.

395.9

A PROPOSED MIGRATORY ROUTE FOR LUTEINIZING HORMONE-RELEASING HORMONE (LHRH)-IMMUNOREACTIVE NEURONS FROM THE MEDIAL OLFACTORY PLACODE TO THE FOREBRAIN IN THE MOUSE. A STUDY USING TRITIATED THYMIDINE AND LHRH IMMUNOCYTOCHEMISTRY. Marlene Schwanzel-Fukuda and Donald W. Pfaff. Rockefeller University, New York, N.Y. 10021.

Swiss mice (two in each age group) were injected with tritiated thymidine (5 uCi/gram of body weight) on day 11, 14, 16 or 18 of pregnancy. Fetal brains were collected 1 hour later and processed for LHRH immunocytochemistry and autoradiography. LHRH-immunoreactivity was first detected in a few cells within the epithelium of the medial olfactory placode at 11 days of gestation. From days 14 to 18, cords of LHRH cells were seen in the placode, on the nasal septum in company with the nervus terminalis and vomeronasal nerves, and in the ventromedial forebrain caudal to the olfactory bulb. Some of these cells were associated with the nervus terminalis, but others (the majority) arched into the septal and preoptic areas of the developing brain. This finding, as well as relative numbers of immunolabeled cells at different ages, supports the notion of a migratory route. Observations of autoradiographic sections showed no thymidine labeling of LHRH cells: the cells of the placode appeared to have undergone division earlier. The nervus terminalis, a derivative of the medial olfactory placode, may serve to guide at least one population of LHRH cells into the forebrain. Supported by NIH grant NS 19662 and funds from the Whitehall Foundation (M.S.-F.).

395.10

NEUROPEPTIDE Y-IMMUNOREACTIVE NEURONS IN THE CORPUS CALLOSUM. B.Y.Kim*, T.Duong and A.B.Scheibel. UCLA Departments of Anatomy, Psychiatry and Brain Research Institute, Los Angeles, CA 90024.

We studied the morphology, distribution and number of neuropeptide Y-immunoreactive (NPY-IR) neurons in the corpus callosum of male and female Sprague-Dawley rats. Cryostat sections (30-50 μm) cut in the coronal, sagittal and horizontal planes were processed by peroxidase-antiperoxidase immunocytochemistry. The corpus callosum in the rat was divided into an anterior and posterior portion using the anterior border of Ammon's horn as the delineating landmark (2mm posterior to bregma). Comparisons were made between anterior and posterior portions of the corpus callosum, the right and left hemispheres, and between the male and female rats. NPY-IR neurons in the rat corpus callosum are small or medium-sized bipolar and multipolar neurons (7-15 μm in diameter). The highest concentration was observed in the forceps minor of the corpus callosum. The anterior portion of the rat corpus callosum contained about twice the number of NPY-IR neurons as the posterior portion. **There was no significant morphological, distributional or numerical difference between hemispheres nor between male and female rats.** We are presently investigating NPY-IR neurons in the corpus callosum of cats and humans. The morphology and size of the NPY-IR neurons appear similar to those in the rat. The distribution of NPY-IR neurons in the human corpus callosum appears sparse.

395.11

TACHYKININS AND THEIR BINDING SITES IN THE INTERPEDUNCULAR NUCLEUS (IPN) OF THE RAT. S. Brown*, T.C. Eckenrode and M. Murray. Medical College of Pennsylvania, Phila., PA 19129.

Tachykinins, identified by an antibody that recognized both SP and SK, and the binding sites for SP and SK are densely concentrated in the habenulo-interpeduncular system. Tachykinins are synthesized in the medial habenulae and transported to the lateral and rostral subnuclei of the IPN. The habenular tachykinin system shows plasticity in response to lesions of the contralateral habenular projection. Intrinsic tachykinin-containing interneurons are present in the ventral division of the rostral subnucleus of the IPN and tachykinin of non-habenular origin is also present in the central and dorsal subnuclei. An antibody specific to SK shows a distribution of habenular and non-habenular staining identical to that of the antibody which recognized both SP and SK, this was confirmed by absorption controls, and the same plasticity was demonstrated using the SK antibody. The SP and SK projections to the IPN appear to be quantitatively and qualitatively very similar. Binding sites for SP and SK were visualized using 125I-SP and 125I-eledoisin. SP binding sites were sparce and confined to the central and rostral subnuclei. The eledoisin binding sites were much denser than the SP binding sites but were also concentrated in the subnuclei which receive only non-habenular tachykinin input. The density of the tachykinin binding sites therefore differ but their patterns are similar and are matched with non-habenular but mismatched with habenular sources of tachykinins.

Supported by NIH grant NS16556.

395.12

IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF THYROTROPIN-RELEASING HORMONE PRECURSOR (pro-TRH) IN THE RAT BRAIN. N. Liao*[1], M. Bulant*[2], H. Nicholas*[2], H. Vaudry*[2] and G. Pelletier[1], [1]Mol. Endocrinol., CHUL, Québec, Canada and [2]Lab. Endocrinologie, Univ. Rouen, France.

In order to study the ultrastructural localization of pro-TRH, we have raised antibodies against a cryptic fragment corresponding to the sequence [Phe178-Glu199] of the precursor. Using preembedding immunostaining, we have investigated two groups of neurons already known to contain pro-TRH: one located in the hypothalamic paraventricular nucleus (HPV) and another one in the raphe nucleus. In the positive neurons of HPV only dense core vesicles (80-100 nm in diameter) which were very numerous were labelled. Immunostained cells, dendrites and endings were frequently observed in contact with unidentified elements. In the median eminence, numerous free endings containing labelled dense core vesicles were detected. In the raphe nucleus a different pattern of labelling was observed. The positive neurons contained only a very few dense core vesicles (60-80 nm in diameter) which were all labelled, the most proeminent labelled organelle being the Golgi apparatus. Stained cell bodies and dendrites were very frequently seen in contact with unlabelled endings. These results suggest that pro-TRH and/or fragments of pro-TRH could play a neuromodulator/neurotransmettor role in different brain areas and could also be released into the pituitary portal plexus. They also demonstrate that pro-TRH can be differently processed by neurons which have different functions.

395.13

EFFECT OF EXTRINSIC DENERVATION ON CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITY (CGRP-IR) IN THE RAT HEPATOBILIARY SYSTEM. L. Goehler and C. Sternini. CURE & Depts. of Psych., Med. & Anat., UCLA, LA, CA 90024.

CGRP-IR is localized in nerve fibers innervating the rodent hepatobiliary system. These fibers are abundant in the biliary pathway, whereas in the liver they are usually restricted to the interlobular space in association with the portal triad. A rich CGRP innervation is also associated with the vena porta. In this study we have investigated the effect of chemical and surgical denervation on the CGRP-IR fibers innervating the hepatobiliary system of the rat by means of immunohistochemistry and radioimmunoassay (RIA). Neonatal treatment with the sensory neurotoxin capsaicin caused a dramatic decrease, and often a complete elimination of the CGRP-IR fibers innervating the biliary tree and hepatic vasculature as well as a marked decrease of those innervating the vena porta. Celiac and superior mesenteric ganglionectomy gave similar results as capsaicin treatment, whereas bilateral vagotomy did not modify the CGRP-IR. RIA studies demonstrated that both capsaicin treatment and ganglionectomy significantly decreased CGRP content in the vena porta and biliary ducts (up to 60 and 85%, respectively). These results suggest that the CGRP innervation of the hepatobiliary system mainly originates from extrinsic, sensory neurons which are likely located within spinal ganglia.
Supported by NIH grant DK38752 and SKB Fellowship.

395.14

DISTRIBUTION OF CHOLECYSTOKININ (CCK)-LIKE IMMUNOREACTIVITY IN THE BRAIN OF THE LITTLE BROWN BAT (MYOTIS LUCIFUGUS). L.K. Laemle and J.R. Cotter. Dept. of Anatomy, New Jersey Medical School, Newark, NJ 07103 and Dept. of Anatomical Sciences, SUNY Buffalo, Buffalo NY 14214.

CCK-like immunoreactivity was localized in the brain of the little brown bat using light microscopic immunocytochemistry. Tissue was fixed in Bouins solution, embedded in paraffin, and sectioned at 10 to 12um. Two primary antisera (Incstar, and Peninsula) and two staining procedures (PAP and avodin biotin) were used. Immunoreactive fibers traveled in three major pathways: median forebrain bundle, dorsal longitudinal fasiculus, and mammillary peduncle. Particularly dense terminal plexuses were formed in the medial septal and dorsal hypothalamic areas, medial and basal amygdaloid nuclei, dorsomedial, parabrachial, and suprachiasmatic nuclei, area postrema and nucleus tractus solitarius. Immunoreactive perikarya were present in the olfactory, amygdaloid, interpeduncular, dorsal tegmental, and suprachiasmatic nuclei; medial preoptic and anterior hypothalamic areas, hippocampus and cerebral cortex.

395.15

LOCALIZATION OF NEUROPEPTIDE Y mRNA IN CHICK BRAIN USING IN SITU HYBRIDIZATION. W.J. Kuenzel, D. Larhammar, J.B. O'Neill, R.J. Milner and J.M. Hill. Dept. of Poultry Sci., Univ. of Maryland, College Pk., MD 20742; Scripps Clinic and Res. Found., La Jolla, CA 92037; and Sect. on Brain Biochem., NIMH, ADAMHA, Bethesda, MD 20892.

Neuropeptide Y (NPY) is a very abundant peptide in the mammalian brain and significantly affects several basic physiological functions, particularly food intake. The peptide has also been shown to effect hyperphagia in chicks. Recently a cDNA clone for chick NPY has been isolated and sequenced (Larhammar, Unpublished). An in vitro transcription system was used to produce single stranded RNA probes. Antisense (experimental) and sense (control) chick NPY riboprobes were labeled with S^{35}. In situ hybridization was conducted using three chicks two weeks of age. They were deeply anesthetized with Chloropent and perfused via the heart with physiological saline. Brains were then quickly removed and frozen in dry ice. Two brains were sectioned in a cryostat at 30 um for in situ hybridization while one had its hypothalamic region dissected for later RNA extraction. A set of slides were treated with RNase to serve as additional controls. Extracted chick brain RNA was analyzed on Northern blots with one of the labeled antisense NPY riboprobes. NPY mRNA was observed in cells of the preoptic periventricular nucleus, the periventricular hypothalamic nucleus, paraventricular nucleus, median eminence, and nucleus of the basal optic root. To date the neural structures identified in the chick to contain NPY mRNA have also been shown by immunocytochemistry to contain NPY-like immunoreactivity.

395.16

CARNOSINE AND ANSERINE LOCALIZATION IN NERVE AND MUSCLE OF RODENTS AND BIRDS. S. Biffo* and F. L. Margolis. Roche Inst. Molec. Biol., Nutley, N.J. 07110

Carnosine (C) is present in olfactory receptor neurons. Two related dipeptides, homocarnosine (H) and anserine (A) are chemically found in excitable tissues (retina, CNS, muscle) but their cellular location is unknown. We raised antisera against C,H, and A. After purification we obtained antisera able to discriminate C from A, but not H from C. C co-localizes with olfactory marker protein in olfactory neurons of mouse and rat. In the mouse C-like IR is observed in an astrocyte population. Analysis suggests that the IR is due to H (Br.Rsch.94,75(1975). This is consistent with the ability of CNS glia to synthesize dipeptides (Bauer et al.J.B.C.257,3593(1982). In chick C-like IR is limited to olfactory nerve axons and terminals. A-like IR is present throughout the CNS in astrocyte-like cells consistent with HPLC evidence for A in chick CNS (Neurochem.Intl.6,207(1984). Thus, in vertebrate CNS C occurs in olfactory neurons but A and H occur in non neuronal cells. Striated muscle of many species contain high levels of C and A. They differ in ontogeny, halflife and proportions in different muscles. Initial results with these antisera indicate that the ratio of C to A varies in different fibers of the same muscle. This novel observation suggests that the amount of C and A may be functionally related to the different metabolic properties of individual muscle fibers.

395.17

BURSICON-LIKE ACTIVITY IN THORACIC PERIPHERAL NEUROSECRETORY CELLS OF ADULT CRICKETS. H.W. Honegger* and I. Garcia-Scheible* (SPON: J. Tautz). Inst. Zoology, TU Munich, D-8046 Garching, Germany.

In insects, neurosecretory neurons have been found not only in the CNS but also in the periphery along nerves. As yet no specific function or defined products could be attributed to such peripheral neurosecretory neurons (PNN).

We have identified a group of PNN in the prothoracic segment of adult crickets which, together with their processes, contains a protein with bursicon-like activity. The processes are located in the sheaths of all anterior nerves of the prothoracic ganglion and in the prothoracic median nerve, a neurohemal organ. A homologous group of PNN in the mesothorax also exhibits bursicon-like activity.

Bursicon was first described by Cotrell (1962) and Fraenkel and Hsiao (1965) as a neurohormone that triggers sclerotization of the insect cuticle. It is present in central neurons of abdominal ganglia in the tobacco hornworm (Taghert and Truman, 1982). The unexpected discovery of a protein with bursicon-like activity in PNN of adult crickets suggests that this product may trigger cuticle sclerotization also in adults. Since it is released along nerves it may have an additional function in the CNS. (Supported by DFG).

395.18

SOMATOSTATIN IMMUNOREACTIVITY IN THE BRAIN OF A GYMNOTID TELEOST FISH. E. Sas and L. Maler. Fac. Health Sci., Dept. Anatomy, Ottawa, Canada. K1H 8M5

The localization of somatostatin immunoreactive (SS ir) perikarya and fibers in Apteronotus leptorhynchus, was studied by immunohistochemistry, using an antibody against somatostatin, kindly provided by Dr. J. C. Brown (UBC).

The largest populations of SS like ir cells were observed in the diencephalon, followed by the telencephalon and rhombencephalon, and a scant amount in the mesencephalon. **I.** Telencephalic ir cells were located mainly in the ventral subdivisions, N. taenia, medial forebrain bundle, entopeduncular nuclei. **II.** Diencephalic cell groups included: the suprachiasmatic N., N. preopticus periventricularis posterior, N. anterior periventricularis, N. pretectalis, glomerular complex, central posterior N., periventricular N. of posterior tuberculum, interphase between the two latter nuclei, interphase of thalamus/hypothalamus, N. tuberis lateralis, dorsal, ventral and lateral hypothalamus, central N. of inferior lobe, N. recessus lateralis and ventral thalamic nuclei. **III.** Mesencephalic SS ir cells were seen in optic tectum, torus semicircularis and interpeduncular nucleus. **IV.** Rhombencephalic ir cells were present in the central gray, paracommissural region, descending trigeminal tract, electrosensory lateral line lobe, reticular formation, vagal N., octaval nuclei. These results will be correlated with those reported in other species, and their possible relevance in the visual and electrosensory systems will be discussed.

395.19

GALANIN-LIKE PEPTIDE IN THE TELEOST SPINAL CORD: DISTRIBUTION AND NOVEL RESPONSE TO INJURY. E. E. Black and R. L. Parsons. Dept. of Anatomy and Neurobiology, Univ. Vermont Coll. Med., Burlington, VT. 05405.

The distribution of a galanin-like peptide in the spinal cord of the molly, Poecilia latipinna, was investigated with immunohistochemical techniques. A dense galanin-like immunoreactivity (GAL-LIR) was present through the length of the spinal cord. Fine varicose fibers coursed longitudinally in the gray matter, and some fibers traversed the neuraxis. Relatively few fibers were seen in the white matter. Transection of the spinal cord at the level of the sixth preterminal vertebra resulted in the disappearance of all GAL-LIR both rostral and caudal to the lesion. In some fish this disappearance was complete as early as 2 days post lesion, and was complete in all fish by 7 days post lesion. By 30 days after transection a heavy GAL-LIR reappeared in some fish at levels rostral to the lesion. Transection of the cord at levels rostral to the sixth preterminal vertebra or at the level of the terminal vertebra also resulted in complete disappearance of GAL-LIR throughout the spinal cord. These studies indicate that a galanin-like peptide is heavily distributed in the gray matter of the teleost spinal cord. Further, injury to any part of this system causes the coordinate loss of GAL-LIR throughout the spinal cord. This response is markedly different from descending putative transmitter pathways (for example, 5-HT, NE,or NPY) we have identified in the fish spinal cord. Supported by PHS R01 19880.

395.20

IMMUNOHISTOCHEMICAL SEARCH FOR SUBSTANCE P, SEROTONIN, CALBINDIN AND LHRH IN THE PRIMARY OLFACTORY SYSTEM OF A TELEOST FISH. J.P. Denizot°, T. Szabo, S. Bläher°, M.Véron-Ravaille°, D. Rouilly°(SPON: A.Mallart), Dept.Neurophysiol Sens, Lab.Physiol.Nerveuse, CNRS, F 91190 Gif sur Yvette.

Substance P, considered as a transmitter or modulator of the nociceptive system, was also revealed in the olfactory bulb of a mammal (Kream RM et al.,JCN, 222:140,1984) and recently in the primary olfactory system of the teleost gymnotids (Szabo T et al., Neurosc.Lett.,81:245,1987).Standard immunohistochemical treatment of paraffin sections with anti-substance P antiserum (1/8000) shows an intense sP-like immunoreactivity (SPLI) in the olfactory epithelium, nerve and olfatory bulb as well as in several tel- di- and mesencephalic areas. Section of the olfactory nerve causes a reduction (in 15 d) or complete depletion in 35d, in all these sites. Identical depletion is obtained 15 d after application of capsaicin (sP antagonist) to the olfactory nerve for 15 min. In contrast, application of colchicin (25%) to the olfactory nerve for 24h produces no depletion of SPLI. Anti-5HT antiserum (1/1000) labeled the primary olfactory projection in the olfactory bulb but not in the other brain areas. No immunoreactivity was obtained with anti-calbindin (CaBP28K) antiserum (provided by M Thomasset). Anti-LHRH antiserum labeled the olfactoretinal ganglion located at the anterior basal part of the olfactory bulb but not the olfactory system.
Supported by the French Medical Research Foundation.

395.21

ULTRASTRUCTURAL LOCALIZATION OF SUBSTANCE P-LIKE IMMUNOREACTIVITY IN THE BRAIN OF LIMULUS. J. K. Johnson and S. C. Chamberlain. Inst. Sens. Res., Syracuse Univ., Syracuse, NY 13244-5290.

Light and electron microscope studies have shown the corpora pedunculata (CP) in Limulus to be synaptically complex and morphologically diverse (Fahrenbach, Tiss. & Cell, 9:157-166, 11:163-200). These anatomical studies have been complemented and extended with immunohistochemical methods in a survey of neuropeptide activity in Limulus brain including a positive reaction for substance P-like immunoreactivity (SPLI) (Chamberlain and Engbretson, J. Comp. Neurol., 208:304-315). Here we report the first demonstration of SPLI in the brain of Limulus at the ultrastructural level.

In the present study a monoclonal antibody against substance P raised in rat (Accurate Chemical) was used to bind substance P-like antigenic sites. Visualization of labelled sites was effected by indirect enzymatic staining with the PAP/DAB procedure (Eldred *et al.*, J. Histochem. & Cytochem., 31:285-292). This technique was used to identify neuron types in the CP that had a substance P-like positive reaction. Results suggest that only the type D afferent fibers possess SPLI in the Kenyon cell layer. In contrast possible type E afferent fibers display a somewhat weaker label in the peduncles and regions of Kenyon cell telodendra, often in close proximity to more heavily labelled type D afferent fibers.

These results are an additional line of evidence suggesting the presence of substance P in Limulus. This technique allows the specific neural circuitry of substance P-like fibers to be elucidated from populations of neurons containing other neuroactive compounds. Continued application of this technique should lead to a detailed understanding of the specific connectivity in the brain and lateral eye of Limulus at the EM level. Supported by EY03446 and EY06064.

395.22

LOCALIZATION OF LEU-ENKEPHALIN-LIKE IMMUNOREACTIVITY IN THE BRAIN OF *LIMULUS POLYPHEMUS*. T. J. Lewandowski*, A. M. Strong*, and S. C. Chamberlain. Institute for Sensory Research and Departments of Bioengineering and Biology, Syracuse University, Syracuse, New York 13244.

Antibodies raised in rabbit against Leu-Enkephalin-bovine thyroglobulin glutaraldehyde conjugate (Incstar) were used to label immunoreactive neurons in the brain of *Limulus polyphemus*. Bound primary antibodies were visualized using FITC labeled secondary antibodies. Labeled sections were photographed using epifluorescence illumination. We present here those neurons displaying Leu-Enkephalin-like immunoreativity using the nomenclature of the *Limulus* brain atlas (Chamberlain and Wyse, *J. Morph.* **187**:363).

Small-diameter fibers were sparsely labeled in the laminar neuropil and richly labeled in the medullar neuropil. Dense populations of small-diameter and large-diameter fibers were labeled in the mottled and large fiber layers of the central body neuropil respectively. A moderate population of small-diameter, varicose fibers labeled in the central neuropil of the brain.

Only two neuronal groups contained labeled somata. Approximately 20 somata labeled in the ventral region of the medullar group, adjacent to the ventral poles of the central body and the optic tract. Two groups of approximately 4 and 8 somata labeled in the dorsal region of the dorsal medial group. Supported by NIH grants and the Department of Bioengineering.

395.23

AN ATRIAL NATRIURETIC-LIKE SUBSTANCE IN THE MARINE MOLLUSK APLYSIA. V.F. Castellucci and J. Gutkowska*. Neurobiology Lab., IRCM Clinical Research Institute of Montreal, Montreal, CANADA, H2W 1R7.

The atrial natriuretic factor (ANF) is a member of a family of peptides that have been first isolated and purified from mammalian atria. It is also detected in plasma, in the peripheral and central nervous systems of vertebrates. Since ANF seems to be related to the water balance and the cardiovascular system of an organism, we were curious to see if this peptide could be detected in APLYSIA and what was its biological role.

We were able to detect ANF-like immunoreactivity in the hemolymph of APLYSIA (5-10 pg/ml); the hemolymph extract had an HPLC profile similar to the ones obtained from vertebrates with a prohormone and a circulating hormone peaks. Significant immunoreactivity was also detected in various organs, such as the heart, the aortae, the gametolytic gland, the salivary gland and the central nervous system. The immunoreactive ANF could displaced synthetic ANF (up to 100%) in a radioreceptor assay procedure. Supported by M.R.C. MA-10047.

395.24

IMMUNOHISTOCHEMICAL STUDY ON VASOACTIVE INTESTINAL POLYPEPTIDE IN THE BUCCAL GANGLION OF APLYSIA CALIFORNICA AND PLEUROBRANCHAEA CALIFORNICA. S. Soinila and G.J. Mpitsos. Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

Vasoactive intestinal polypeptide (VIP) is a putative neurotransmitter/neuromodulator in several vertebrate and invertebrate species. The present study was undertaken to define simple experimental models that would allow us to examine the role of VIP in the nervous systems of marine molluscs.

Each buccal hemiganglion contained on the coelomic (caudal) surface a single 100 μm VIP neuron. A cluster of small VIP cells (30 μm) was observed constantly near the outlets of buccal roots 1 and 2. These neurons projected into the buccal muscles. Another group of small VIP cells was located ventrally to the commissure on each side. In Aplysia, but not in Pleurobranchaea, the buccal (rostral) surface contained a large group of VIP neurons which extended from the lateral S-region to the central region.

These results provide a morphological basis for comparative studies on the effects of VIP on Aplysia and Pleurobranchaea feeding behavior. Of particular interest is to use such biochemical and neuroanatomical methods as probes to identify groups of neurons whose group dynamics can then be analyzed to determine the principles of self-organizing activity and of the role of variability in it (e.g. see Mpitsos et al., 1988, these proceedings, and Mpitsos, G. J., and Cohan, C. S., J. Neurobiol., 17: 517, 1986).

This study was supported by the grant AFOSR-86-0076 to G.J.M.

RETINA V

396.1

THE QUANTAL NATURE OF SYNAPTIC TRANSMISSION FROM PHOTORECEPTORS TO BIPOLAR CELLS. *B.R. Maple and F.S. Werblin, Graduate Group in Neurobiology, University of California, Berkeley, CA 94720*

Bipolar cells of the Tiger Salamander retina were voltage clamped with patch electrodes in light adapted retinal slices. Transmission from photoreceptors was elicited by depolarizing rods with a second patch electrode, and bipolar cell dendritic conductances were also activated directly by pressure ejection of glutamate at the outer plexiform layer.

In some OFF bipolar cells, rod depolarization elicited responses containing miniature excitatory synaptic currents. These quantal events reversed near -5 mV, the reversal potential for the dendritic glutamate response. They represented a peak conductance change of about 150 pS, with a rise time of 1 msec and a falling time constant of about 3 msec. Insensitive to tetrodotoxin, and seen in cells with transected axons, these events may reflect the release of single transmitter vesicles as are found at photoreceptor ribbon synapses.

In other OFF bipolar cells, rod depolarization elicited a similar conductance change with no discernable quantal component. These responses also reversed near -5 mV, so they were probably not electrotonically decremented or filtered. They may reflect a form of nonquantal, nonvesicular release possibly occuring at photoreceptor basal junctions. This transmission, as well as quantal transmission, was abolished by lowering extracellular calcium to 1 uM or substituting cobalt for calcium.

No corresponding quantal events were resolved in ON bipolar cells. Responses both to rod depolarization and to glutamate (conductance decreases associated with a reversal potential near 0 mV) tended to be very small, suggesting that rod to ON bipolar cell transmission is turned off postsynaptically in light adapted slices. Relatively large light responses obtained in these cells suggest there is a cone transmitter that is not glutamate-like.

396.2

GLUTAMATE RESPONSES IN SOLITARY BIPOLAR CELLS FROM SALAMANDER RETINA. *A.A. Hirano, J.A. Hirsch, and P.R. MacLeish.* Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

We have studied the currents evoked by L-glutamate and D,L-APB in single isolated bipolar cells enzymatically dissociated with papain or dispase/ collagenase from the adult tiger salamander retina. The cells were maintained in serum-free media at 12°C for up to several weeks before use, with no obvious change in response properties. During recording, the cells were superfused with a simple salt solution. In bipolar cells, identified by their characteristic morphology and I-V curve, glutamate-induced currents were recorded using whole-cell patch clamp recording techniques. Electrodes had a tip diameter of about 1 μM and contained (in mM): D-aspartate,100; NaCl,12; NaH_2PO_4,2.5; $MgCl_2$,1; EGTA,0.05; Na_2 ATP,1; HEPES,2; sucrose,20; adjusted to a pH 7.1 with KOH. The pharmacological agents were applied by pressure ejection from a second pipette positioned near the cell. We studied 41 bipolar cells of which 14 showed no response to L-glutamate (0.1-1 mM), and the rest of the cells fell into two classes. 9/22 of the papain- and only 1/19 of the dispase/ collagenase-dissociated cells responded with a large inward current that reversed around +10mV; in addition, 3/3 of papain-dissociated cells tested showed inward currents in response to kainate (25-50 μM). In contrast, 4/22 of the papain- and 13/19 of the dispase/collagenase-dissociated cells showed a small outward current with a reversal potential of about -70mV. Similarly, 4/4 of the dispase/collagenase- dissociated cells tested showed outward currents to APB (2-amino-4-phosphono butyrate, 50 μM), a known agonist for ON-bipolar cells (Slaughter & Miller, J.Neurosci., 5:224, 1985). Multipolar cells did not show this difference depending on dissociation procedures; all of the responding cells (27/33) gave a clear inward current in response to glutamate (0.1-0.5 mM). The glutamate-evoked currents of opposite polarity we observed in the bipolar cells may underlie the physiological basis of the ON- and OFF- responses in these second-order neurons. [Supported by the Lucille P. Markey Charitable Trust, NIH EY05201, the Klingenstein Fund, and an Javits Center Award (NS-22789).]

396.3

PERMEABILITY OF GLUTAMATE-GATED CHANNELS IN RETINAL BIPOLAR CELLS. T.A. Gilbertson,*[1] R.P. Scobey[2] and M. Wilson[1] (SPON: F.A. Gorin) [1]Dept. of Zoology, [2]Dept. of Neurology, Univ. of California, Davis, CA 95616.

Retinal bipolar cells make up the second neuron layer in the visual system and provide the main link between the inner and outer retina. The probable transmitter operating at the photoreceptor-bipolar cell synapse is L-glutamate (Glu).

In the present study, we have investigated current responses to Glu and its structural analogues in isolated bipolar cells from the larval tiger salamander (*Ambystoma tigrinum*) retina under whole-cell patch clamp conditions. The cells examined were apparent hyperpolarizing bipolar cells on the basis of a conductance-increase response to Glu. The channels gated by Glu and kainate (KA) activate conductances with similar ionic selectivities. The relative permeability (P) estimates for these channels are: P_K = 1, P_{Na} = 1, P_{Ca} = 3, P_{Mg} = 0.25, $P_{N\text{-methyl-D-glucamine}}$ = 0.15, P_{Cl} = 0. The Glu analogue N-methyl-D-aspartate (NMDA) causes no conductance change in the bipolar cells. The Glu- or KA-induced current is not blocked by external Mg^{++}.

Greater than 50% of the Glu-induced current desensitizes within 20 msec of the onset of application, at Glu concentrations greater than 100 uM. Desensitization is voltage-dependent, being more pronounced at larger positive and negative potentials. The KA response does not desensitize at any concentrations tested.

396.4

ELECTRICAL COUPLING BETWEEN BIPOLAR CELLS IS UNLIKELY TO ACCOUNT FOR THE LARGE RECEPTIVE FIELD CENTERS OF THESE CELLS. S. Borges and M. Wilson. Dept. of Zoology, Univ. of California, Davis, CA 95616.

When mapped with a small, dim spot of light, the receptive field centers for bipolar cells in the dark adapted retinae of tiger salamanders have a diameter between 374 and 662μm (Borges, S. and Wilson, M., J. Neurophysiol., 58:1275,1987). This is much larger than the dendritic arborization of these cells. What accounts for the large receptive fields of these cells? Characteristically, distant spots of light evoke bipolar cell responses with very long (up to 940 msec) latencies. Modelling the bipolar cells as a two dimensional network, we show that electrical coupling between bipolar cells cannot account for the observed latency. Similarly we show that reciprocal, delayed, chemical synapses between bipolar cells are an unlikely explanation. Another cell type, possibly an amacrine cell, is likely to be involved in enlarging bipolar cell fields. We have observed clear P.S.P's, presumably resulting from amacrine cell spikes, in some bipolar cells.

396.5

APB INCREASES ELECTRICAL COUPLING BETWEEN HORIZONTAL CELLS. C.-J. Dong* and J.S. McReynolds. Department of Physiology, The University of Michigan, Ann Arbor, MI 48109.

2-amino-4-phosphonobutyrate (APB) is an agonist at a unique type of glutamate receptor thought to be restricted to depolarizing bipolar cells in the retina. We find that APB also has markedly different effects on the responses of mudpuppy horizontal cells (HC) to central and peripheral illumination. 5 μM APB caused a decrease in HC responses to small centered spots and an increase in HC responses to concentric annuli, indicating an increase in coupling. APB also caused a hyperpolarization in darkness and increased the amplitude of responses to diffuse illumination, neither of which can be explained by an increase in coupling. All of these effects were opposite to those of dopamine in fish retina (see Dowling, TINS, Apr. 1986). The effects of dopamine on mudpuppy HC were qualitatively similar to those in fish, but much weaker and often absent, even at concentrations as high as 2 mM. The above results suggest that in mudpuppy APB acts either directly on HC in a manner opposite to that of dopamine in fish, or decreases the tonic release of a transmitter with an action similar to that of dopamine. We also find that APB greatly enhances surround responses in some ganglion cells; this may be due to its effect on HC coupling. The interpretation of APB-induced changes in responses of more proximal neurons should therefore take into account its effect on lateral interactions in the outer retina. Supported by NIH Grant EY01653.

396.6

CORTICOTROPIN-RELEASING FACTOR-INDUCED CURRENTS IN ACUTELY DISSOCIATED RAT RETINAL GANGLION AND BIPOLAR CELLS. Hermes H. Yeh, Department of Neurobiology and Anatomy, Univ. Rochester School of Medicine, Rochester, NY 14642.

In the rat retina, corticotropin releasing factor(CRF)-like immunoreactivity has been localized to a subpopulation of amacrine and displaced amacrine cells. In this preliminary study, whole-cell patch clamping was used to determine whether CRF could elicit current responses in isolated ganglion and bipolar cells.

Ganglion cells were identified by the presence of rhodamine-labeled microspheres injected into the superior colliculi 2-4 days prior to enucleation. In the physiological potential range, pressure-applied pulses (50 ms-3 s) of CRF (50-100 uM) induced prompt inward currents in 3 of 5 ganglion cells. I-V relation showed a reversal potential near 0 mv and a change in CRF-induced current that is essentially linear, with a slight tendency towards rectifying at positive holding potentials. In 4 cases, prolonged and continuous application of CRF partially suppressed both inward and outward currents evoked by depolarizing voltage steps.

Many bipolar cells retain their characteristic morphology following dissociation. Held at the resting potential range, CRF induced inward (2 cases) or outward currents (3 cases) in bipolar cells. Both types of currents reversed between 0 mv and +20 mv. Whether these responses reflect differential effects of CRF on presumed hyperpolarizing and depolarizing bipolar cells has yet to be determined.

In summary, CRF exerts postsynaptic effects on retinal neurons consistent with its anatomical disposition and thus may be considered as an amacrine cell transmitter candidate. Studies are ongoing to determine the ionic selectivity underlying the CRF-induced whole cell currents.

Supported by PHS grant NS24830 and the Rochester Eye Bank.

396.7

A CELLULAR MODEL OF SELECTIVE ATTENTION Z-H. Pan* and M.M. Slaughter, Dept of Biophysics, SUNY, Buffalo, NY 14214

Recording intracellularly in the superfused tiger salamander retina, we have found that baclofen, a GABA-B receptor agonist, makes amacrine and ganglion cells respond more transiently to a step of light. In third order neurons that normally respond transiently, baclofen application enhances the amplitude of these transient responses. But in cells that normally respond in a sustained manner to a step of light, baclofen application often suppresses the sustained responses and the cells begin to respond like transient neurons. Since tonic and phasic responses can carry very different information, the action of this GABA analog suggests that response characteristics of retinal neurons could be under synaptic control. To test this, we looked at trigger features that might be induced by baclofen. One stimulus feature that we examined was orientation selectivity, but we found no effect of baclofen on this property. However, when we studied directional selectivity, we found that baclofen did modify the response properties. In some cells that showed no directional selectivity to a moving slit under control conditions, a pronounced directional selectivity became evident after baclofen treatment. This change in response properties may be a form of selective attention. If baclofen's action is related to selective attention, then it should under efferent control. Dr. S. Ball has anatomical evidence of efferent inputs to GABAergic amacrine cells, which could potentially serve this function (pers.com.). NEI EY05725

396.8

N-METHYL-D-ASPARTATE RECEPTORS MEDIATE A SLOW COMPONENT OF THE TRANSIENT EXCITATORY LIGHT RESPONSE OF TIGER SALAMANDER RETINAL GANGLION CELLS. S. Mittman and W.R. Taylor*. Dept. of Ophthalmology, UCSF, San Francisco, CA 94143.

In tight-seal, whole-cell recordings from cells of the ganglion cell layer of tiger salamander retinal slices, we observed both sustained (< 20% of cells) and transient (> 80% of cells) light-evoked excitatory responses due to increases in cation conductances. To characterize these conductances, we blocked contaminating inhibitory responses with 500 nM strychnine and 100 μM bicuculline methobromide.

Two conductances, with different time courses, mediated the transient excitatory response. A fast conductance with a linear current-voltage relationship (I-V) and a reversal potential of ≈0 mV, had a time-to-peak (t_p) of 20 - 50 ms, and a half-decay-time ($t_{\frac{1}{2}}$) of 15 - 80 ms. A second, slow conductance had the same reversal potential, a longer t_p and a $t_{\frac{1}{2}}$ of 250 - 500 ms. The slow conductance had a non-linear I-V with negative slope at potentials more hyperpolarized than -30 mV, typical of conductances gated by N-methyl-D-aspartate (NMDA). The NMDA-receptor antagonist 2-amino-7-phosphonoheptanoate completely and reversibly blocked the slow, but not the fast, conductance at a concentration of 20 μM. Eliminating external Mg^{2+} (usually 1 mM), greatly reduced the voltage-dependence of the slow conductance, as expected of a NMDA-gated conductance, but did not effect the fast conductance. Ionophoresis or bath application of NMDA and kainate (in the presence of 20 μM Cd^{2+}) evoked conductances with I-V's similar to those of the slow and fast conductances, respectively.

These results demonstrate that the transient excitatory response is mediated by a mixed population of receptors. NMDA receptors are the post-synaptic mediators of the slow component of this response, but the pharmacology of receptors underlying the fast component remains undetermined.

396.9

EFFECTS OF MUSCIMOL ON CAT RETINAL GANGLION CELL ACTIVITY. A. W. Przybyszewski, O.-J. Grüsser, M. Hagner* and N. Sucher*. Dept. of Physiology, Freie Universität Berlin, Arnimallee 22, 1 Berlin 33, Germany. [SPON: European Brain and Behavior Society].

Ibotenic acid (IBO) is metabolized into muscimol (MU), a hypothetic GABA-agonist. We investigated the effects of MU injections (0.2-2mg/eye) into the vitreous body on ganglion cell activity evoked by diffuse short flashes and moving contrast gratings. The experiments were performed in pentobarbital anesthetized cats. Single ganglion cell activity was recorded by means of microelectrodes from optic tract axons.

20-40 min after injection the spontaneous flash-evoked activity of off-center ganglion cells was reduced and disappeared after 60-90 min. In on-center ganglion cells the spontaneous activity disappeared about 30 min after MU injections and the light flash responses were reduced to 2-5 action potentials per flash. The ganglion cell responses to moving gratings were reduced 20-30 min after MU injections and disappeared 10-40 min later. The relative efficiency of MU depended on spatial frequency of the grating and on drift angular velocity. Thus spatial and/or temporal factors are variables in the MU-effects on ganglion cell activity. The effect of MU on retinal ganglion cell activity is compared with that of IBO applied in comparable doses. For both substances no significant differences in the in the sensitivity of latency class I and II neurons were found.

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396.10

RESPONSES OF CAT RETINAL GANGLION CELLS TO EYEBALL DEFORMATION DURING DIFFERENT STATES OF DARK ADAPTATION. M. Hagner*, A.W. Przybyszewski and O.-J. Grüsser. Dept. of Physiology, Freie Universität, Arnimallee 22, 1000 Berlin 33, Germany.

Eyeball deformation presumably causes a depolarization of the horizontal cell membrane potential. This effect induces via bipolar cells an activation of on-center ganglion cells (X- and Y-type) and an inhibition of off-center ganglion cells during the deformation. In the present study we examined the effect of dark adaptation on this response pattern. Defined lateral eyeball deformation was applied in total darkness 1 to 40 minutes after the onset of dark adaptation of a previously light-adapted cat retina (1-2 $cd.m^{-2}$). Responses of on-center and off-center ganglion cells were recorded from single optic tract axons.

1) The general response pattern of on-center and off-center ganglion cells was not changed during dark adaptation.

2) During the course of dark adaptation a small decrease in on-center ganglion cell activation relative to the spontaneous activity was observed.

3) Off-center neuron activity increased slightly during dark adaptation, but the inhibiting effect of eyeball deformation remained constant.

4) Our findings indicate that processes involving the receptor outer segments are not implicated in the generation of the retinal ganglion cell deformation responses.

The work was supported in part by a grant of the DFG (Gr 161)

396.11

ARE THERE ON AND OFF ROD BIPOLARS IN THE PRIMATE RETINA? R.P. Dolan and P.H. Schiller, Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA. 02139.

Recent evidence suggests that there may be only one class of rod bipolar cells in the mammalian retina, which hyperpolarize to light (Müller et. al., J. Neurosci, in press). This contrasts with the cone system in which both hyperpolarizing (OFF) and depolarizing (ON) bipolars have been identified. We present support for the contention that rod bipolars are all depolarizing.

Adult rhesus monkeys were trained on a detection task in which they made direct saccades to achromatic stimuli appearing on a CRT; stimuli presented were either brighter (incremental) or darker (decremental) than background. Monkeys were tested under both light- and dark-adapted states. Intravitreal injections of 2-amino-4-phosophonobutyrate (APB) were made into one eye and the monkeys' performance for each eye was assessed independently.

As we have found before, for photopic vision, APB greatly impaired the monkeys' ability to detect incremental stimuli, but had little effect on the detection of decremental stimuli. For scotopic vision, APB eliminated all responses to both incremental and decremental stimuli.

These findings support the existence of a single physiological class of rod bipolars that depolarize in response to light.

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396.12

NON-LINEAR SUMMATION OF M- AND L- CONE INPUTS TO PHASIC GANGLION CELLS OF THE MACAQUE. B.B.Lee, P.R.Martin*and A. Valberg* Max-Planck-Inst. Biophys. Chem. 34 Göttingen FRG.

Phasic ganglion cells in the macaque retina receive combined input from M- and L- cones to both center and surround of their receptive fields. With sinusoidal alternation (flicker) of two lights of different color but having equal luminance, the M- and L- cones can be modulated in antiphase. With a 4° stimulus field at a retinal illuminance of 1400 td, phasic cells responded at twice the flicker frequency for all frequencies between 1 and 20Hz. With different pairs of alternating lights, the amplitude of the frequency-doubled response was directly related to the degree to which M- and L- cones were stimulated in antiphase. This suggests that the response is due to a non-linearity seen on summation of M- and L- cone inputs. The response was largely abolished when a 0.5° field restricted to the center of the receptive field was used, or at low mean luminance levels. The non-linearity thus arises largely in the surround mechanism at high retinal luminances. Some of its characteristics suggested a saturating non-linearity, but detailed examination of the response histograms suggested the mechanism may be more complex.

396.13

SYNTHESIS OF RETINAL GANGLION X AND Y RECEPTIVE FIELDS WITH A COMPUTER CELL PACKING MODEL. A. Moore* and C. Koch (SPON: D. van Essen) . Computation & Neural Systems Program, California Institute of Technology, Pasadena, CA, 91125.

Retinal ganglion (RG) cells are known to have smooth, roughly gaussian excitatory centers, even though the dendritic trees upon which the receptive field (RF) is constructed have large gaps between branches. It is a challenge of neural systems modelling to explain how excitation from cones, as it passes to RG cells via bipolar (BP) and amacrine (AM) cells, is smoothed enough spatially to 'fill in' these gaps (Peichl and Wassle, J. Physiol 262, 1976, p 280). In cat, the classes of BPs that contact on-and off-center X and Y class RG are known but the classes of AM cells that contribute to their RFs are not fully known. By studying which AM cell classes are sufficient to smooth the X and Y RFs, we hope to suggest which AM classes transfer information in the X and Y pathways. Thus, by applying a functional constraint (smoothness) we can make predictions about pathway structure in the retina. Images of cells at 3mm eccentricity are captured with a video camera, converted to scaled binary images, and stored in planes of computer memory. Synthetic spots are placed in the stimulus field: where a spot contacts a cone in the adjacent plane, the cone is excited. Similarly, where excited cones contact a BP input dendrite, the BP cell is excited. The excitations from cones are summed, and the total is passed to the BP output dendrites. This simulates the convergence and divergence of excitation as it passes from the outer to inner plexiform layer. The RG dendrites and soma that contact the output BP dendrites in turn sum the BP excitation. The total excitation at the RG cell due to the cone-BP-RG path is then taken as the RG response to the spot, and is placed in register with the spot in the output (RF) plane. For X RG cells, we have found that AM intermediates are not necessary to satisfy the smoothness constraint for spot stimuli of at least 25 um in diameter. For Y cells, either larger spots or AM intermediates are necessary.

396.14

A NEURAL NETWORK MODEL OF VISUAL RECEPTIVE FIELD REGIONS AS A FUNCTION OF RESPONSE LATENCIES. L. Sun* and E. Micheli-Tzanakou. Dept. of Biomedical Engineering, Rutgers University, P.O.Box 909, Piscataway, New Jersey 08855-0909.

Last year we reported about the spatiotemporal characteristics of the frog ganglion cells (1). It was found that an anticorrelation exists between the number of responses and the latencies. A parabolic relationship was also determined between the latency and the receptive field cluster as a cluster moved toward the surround. In this paper a three dimensional neural network model of the retina has been designed and computer simulations carried out in order to duplicate the experimental results and to further understand the retinal processes. The results show that the responses reach a maximum at the beginning of stimulation and then adapt to a certain level after repeated stimulation. The responses vs. latencies show a nonlinear relationship the same as in the experimental results. If the frequency of stimulation increases then repeated stimuli do not evoke repeated responses. The stimuli used were equivalent to the receptive field organization previously used in our experiments. The implications of this retinal network will be discussed.

396.15

INTERACTION BETWEEN NEUROPEPTIDES AND GLYCINE IN THE AVIAN RETINA. T. Li*, D.M.K.Lam and Y.Y.T. Su. Center for Biotechnology, Baylor College of Medicine. Houston, TX 77030.

Previous studies from this Laboratory have demonstrated the interaction between enkephalins and GABA, glycine or dopamine in the chicken retina (Su et al., Retinal Signal Systems, Degenerations and Transplants, 9:53, 1986). In this communication we present our recent studies on the interaction between somatostatin and glycine, neurotensin and glycine in the chicken retina. Our preliminary results showed that in the presence of 2 μM of somatostatin approximately 32 ± 4% of K^+-induced ^{3}H-glycine release was inhibited, while the same dosage of neurotensin increased ^{3}H-glycine release by 36 ± 2%. This suggested these peptides and glycine are functionally related. The structural basis of these systems was studied using double-label techniques. In addition to those cells which were labelled for each marker alone, some of the cells were found to be double-labelled for both ^{3}H-glycine and somatostatin- or neurotensin-immunoreactivity. The neuropeptide- and glycine-stained processes, which may have originated from single- or double-labelled cells, overlapped extensively in the inner plexiform layer. The interaction between these systems may be confined to this layer, where somatostatin may function as a presynaptic inhibitor and neurotensin as a presynaptic activator of glycine accumulating terminals.

396.16

MUSCARINIC RECEPTORS AND SECOND MESSENGER SYSTEMS IN THE RAT RETINA. S.E. Moroi, Z.-X. Qu*, G.-Y. Le*, M. Hadjiconstantinou and N.H. Neff. Depts. Pharmacology and Pathology, The Ohio State Univ. Col. Med., Columbus, OH 43210

Receptor-mediated transmembrane signaling is modulated by interactions among second messenger systems. We investigated potential interactions among cAMP and phosphoinositide (PI) second messenger systems coupled to muscarinic receptors in rat retina. Maximal stimulation with acetylcholine (ACh) caused a 30% decrease in cAMP and a 100% increase in inositol monophosphate (IP_1). Preincubation with cyclic nucleotide analogs (8-BrcAMP, dibutyryl cAMP, and 8-BrcGMP at 1 mM) and forskolin (10 uM) had no effect on basal or stimulated IP_1 which suggests that cAMP does not modulate ACh-mediated PI hydrolysis. The phorbol ester, PMA, was used to stimulate protein kinase C (PKC). Preincubation with PMA prevented ACh stimulation of IP_1 which is consistent with down regulation of muscarinic receptor function. PMA caused a 65% increase in basal cAMP and attenuated the ACh-mediated inhibition of cAMP. This finding suggests that PMA also causes functional down regulation of muscarinic receptors coupled to cAMP. These preliminary results suggest that PKC modulates muscarinic receptors coupled to both the cAMP and PI second messenger systems.

396.17

GLYCINE STIMULATES CALCIUM INDEPENDENT RELEASE OF ^{3}H-GABA FROM XENOPUS LAEVIS RETINA. John F. Smiley* and Scott F. Basinger, Program in Neuroscience and Cullen Eye Institute Baylor College of Medicine, Houston, TX. 77030

We have previously characterized an interplexiform cell in the Xenopus laevis retina that is labeled by both glycine high affinity uptake and somatostatin antibodies. We are now looking at the function of these two putative transmitters by monitoring the release of retinal ^{3}H-GABA, presumably from horizontal cells. Retinas are incubated 10 minutes in luM ^{3}H-GABA, perfused for 32 minutes, and then drugs are added to the perfusate. In most experiments six consecutive 4 minute pulses of drug are applied, with 10 minute washes between pulses.

Micromolar concentrations of glycine or glutamate are seen to stimulate ^{3}H-GABA release with approximately equal potency. These effects are completely independent of Ca^{2+}, as seen in Ringers with 0 Ca^{2+}/20mM Mg^{2+}. One difference between these responses is that glutamate down-regulates itself, causing substantially smaller responses in consecutive pulses. In contrast the glycine response at first increases, and then only gradually decreases. These patterns of self-regulation are uneffected by Ca^{2+} concentration. The response to glycine is inhibited by strychnine. Potassium also stimulates release, but this is about 75% Ca^{2+} dependent. Somatostatin-14 or -28 have no effect at concentrations of 10nM to 2uM. (Supported by NIH grants.)

396.18

GLYCINE INDUCED REVERSIBLE BLINDNESS IN SHEEP. N. Wright, R. Hill, J. Seggie (SPON: M. Steiner). Departments of Biomedical Sciences, Psychiatry, Medicine and Pathology, McMaster University, Hamilton, Ontario L8N 3Z5.

The use of a 1.5% glycine solution as a bladder irrigant during surgical removal of the prostate has been associated with transient visual impairment. Glycine is thought to be an inhibitory retinal neurotransmitter. Adult female sheep (N=17) were infused with 0, 500, 1000, 2000 or 4000 ml of 1.5% glycine. The volume control was a solution of dextrose and saline. The pupil response to 5 seconds of bright light following dark dilation was used as an index of retinal response. Observations were made at 2, 4, 6, 12, 24, 48, 96 and 192 hours after infusion. Glycine infusion resulting in plasma levels up to 15,000 μmol/L inhibited pupil response to light is a dose dependent fashion. Inhibition of pupil response was paralleled by behavioural indices of visual impairment and blindness but not by changes in plasma sodium, chloride, potassium or osmolarity. The duration of effect was also dose dependent with visual impairment following 4000 ml of glycine being detectable up to 24 hours. Data suggest that glycine inhibits retinal responsiveness to light in a dose dependent and reversible manner.

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396.19

GLYCINE AND GABA ACTION ON THE SCOTOPIC THRESHOLD RESPONSE OF THE CAT ERG. F. Naarendorp, M. Adams*, P.A. Sieving* Kellogg Eye Center, Univ. of Mich., Ann Arbor, MI 48105

The Scotopic Threshold Response (STR) is a component of the electroretinogram (ERG) recorded near rod absolute threshold. The STR appears below PII threshold, in relative isolation from other ERG components. We studied the effects of intravitreal injections of Glycine and Gaba on the STR in the optically intact eye of anesthetized cats. Glycine (10-50 uMole) reduced maximal amplitudes of the STR and PII to 20% and 70% of control values respectively; PII-threshold remained unaltered but the STR was desensitized by one log unit. The photoreceptor a-wave was unaffected. Strychnine (0.1-0.4 uMole) antagonized the effect of Glycine on the STR and PII. Gaba (20-40 uMole) suppressed the STR completely, lowered PII-threshold by 0.5 log units and enhanced its amplitude significantly across the initial 2/3 of its dynamic range without changing Vmax. Bicuculline and picrotoxin prevented enchancement of PII-amplitude by Gaba; they partially blocked the suppressive effect of Gaba on the STR. Recovery from Glycine and Gaba showed a return of PII to control amplitude but an enhancement of the STR. Although both Gaba and Glycine exert a neuromodulatory role on the cells that control STR, they may do so in different ways.

NEI RO1-06094 (PAS)

396.20

DOSE RELATED DIFFERENTIAL EFFECTS OF APOMORPHINE ON THE RABBIT SCOTOPIC ERG. P. Olivier, F.B. Jolicoeur,G Lafond*, A Drumheller and J.R. Brunette*. Departments of Ophthalmology and Psychiatry, Faculty of Medecine, Sherbrooke University, Sherbrooke, Quebec, Canada J1H 5N4.

In order to better understand dopaminergic functions in mammalian retina we initiated a series of experiments in which the effects of several doses of the well known dopamine agonist apomorphine on the rabbit scotopic ERG were examined.

The effects of 0.01,0.1,1.0 mg/Kg of the drug administered intravenously in different groups (N=10) were studied in dark adapted rabbits and compared to a control group of animals (N=10) injected with the same volume of the vehicule solution. ERG parameters investigated included amplitudes and implicit times of A- and B-waves as well as four oscillatory potentials (OP1- OP4) generated by different intensities of stimulation and recorded before and15,30,60,90 and 120 min after injections.

Results indicate that neither amplitudes nor implicit times of A-waves were affected by apomorphine. Interestingly, apomorphine produced dose related differential effects on B-wave amplitudes without affecting its implicit times. The smallest dose (0.01 mg) significantly increased amplitudes at all intensities studied. The intermediate dose (0.1 mg) was without any effects whereas the largest dose (1.0 mg) markedly decreased B-wave amplitudes, an effect more prominent at high intensities of stimulation. It is noteworthy that on oscillatory potentials, apomorphine produced an enhancement of OP2 and OP3 amplitudes only with the intermediate dose (0.1 mg). Other oscillatory potentials were not altered by the drug.

Together, our results constitute experimental evidence that retinal dopaminergic neurons implicated in the generation of the B-wave and specific oscillatory potentials amplitudes possess presynaptic inhibitory receptors which are stimulated by relatively small doses of a dopamine agonist. The fact that A-wave, OP1 and OP4 amplitudes or implicit times of all ERG parameters remained unaffected suggest that retinal dopamine autoreceptors are differentially implicated in retinal response to photic stimulation. Supported by MRC grants MT 2593 and DG 284.

397.1

THE VISUAL WULST MODULATES THE DIRECTIONAL SELECTIVITY OF ACCESSORY OPTIC UNITS IN THE PIGEON. L.R.G. Britto, D. E. Hamassaki*and O.C. Gasparotto*. Dept.Physiol.Biophys,Inst Biomed.Sci., São Paulo State Univ.,05508 São Paulo,Brazil

The visual telencephalon is known to project to accessory optic system (AOS) nuclei. In order to verify the impact of that connection upon the AOS function, we examined the directional selectivity of units within the nucleus of the basal optic root (nBOR) of the pigeon AOS , before and after visual Wulst lesions. Thirty adult pigeons (Columba livia) were urethane-anesthetized and subjected to monocular dynamic photic stimulation. Half such birds previously suffered unilateral or bilateral Wulst ablations. Directional selectivity of nBOR units recorded with tungsten electrodes was then evaluated in terms of a vector analysis. In the normal pigeon, most nBOR neurons displayed preferences to upward-temporal or downward- nasal motion. After ipsilateral or bilateral Wulst lesions, the upward response components were almost absent, with most units now preferring temporal or downward-nasal motion. The contralateral lesions generated, instead, a dramatic reduction in downward responses. Indeed, most units showed in these cases upward-temporal or upward-nasal responses. These data suggest an involvement of the visual Wulst in the elaboration of the sensitivity to vertical stimulus motion of AOS neurons.

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397.2

FOURIER ANALYSIS APPLIED TO VISUAL NEURONS TO DETERMINE DIRECTIONALITY. R. SIMINOFF, UNIVERSITY OF MASSACHUSSETTS AMHERST, MA (SPONSOR J. Desmond)

A neuron displays directional symmetry (DS) if (a) there is a preferred direction (PD) to stimuli moving across the receptive field, (b) there is a null direction (ND) 180 deg out of phase, and (c) responses decrease monotonically and symmetrically from the PD to ND. Degrees of directionality of 110 neurons located in the pretectum of the frog (Fite et al., 1987) using gratings moving at 6, 15 and 25 deg/sec and 8 orientations are evaluated using Fourier Transforms. Practically all units studied show some aspects of directionality and form a continuum from DS to progressively greater asymmetries with non-directionality at the other end. Deviations from DS consist of (a) a ND less than 180 deg out of phase, and (b)responses do not decrease monotonically and/or symmetrically from the PD to ND. Even the most DS neurons do not decrease from the PD to ND as a straight line function. PD's are independent of velocity in 75% of the neurons, and are along the nasal-temporal and vertical axes in 32 and 31% of the neurons, respectively. ND's are 180 and 90 deg out of phase in 30 and 40% of the neurons, respectively. While directionality is independent of background activity, a unit that is "broadly-tuned" can become "narrowly-tuned" if background activity was to increase since the background activity sets the zero level.

397.3

DIRECTION-SELECTIVE CELLS IN THE NUCLEUS OF THE OPTIC TRACT OF THE OPOSSUM (DIDELPHIS MARSUPIALIS AURITA). E. Volchan*, C.E. Rocha-Miranda*, C.Picanço-Diniz*, B. Zinsmeisser*, R.F.Bernardes* and J.G.Franca* (SPON: I.Izquierdo) Inst. de Biofísica, UFRJ, Rio de Janeiro, 21941, Brazil.

Single-unit recordings were performed on eight adult opossums subjected to a midpontine pretrigeminal section. The electrodes were oriented to the brachium of the superior colliculus based on a previous reconstruction of notal--olivary projecting cells. Most of the units showed excitation for random dots stimuli presented to the contralateral eye in a temporo-nasal direction and/or inhibition in the opposite direction. An average of 45% of the units responded to the ipsilateral eye. With two exceptions, wich had the strongest excitatory response at opposite directions for each eye, both eyes showed the same overall pattern of directionality. Inhibiton was stronger than excitation in most ipsilaterally responding cells. Excitatory response to the ipsilateral eye was always weaker than that elicited by the contralateral but in a few cases the ipsilateral inhibitory response was strongest. Many units were excited by stimulus velocities ranging from 0.6 to 150 deg/s. Inhibitory responses were tunned to intermediate spped. The response properties of these units show similarities and discrepancies with other species that can be correlated with the differences in the performance of the optokinetic reflex.

(supported by FINEP and CNPq)

397.4

A QUANTITATIVE ANALYSIS OF THE VISUAL RESPONSE PROPERTIES OF NEURONS IN THE NUCLEUS OF THE BASAL OPTIC ROOT (nBOR) OF THE PIGEON. D.R. Wylie* and B.J. Frost (SPON: M.B. Calford). Dept. of Psychology, Queen's Univ., Kingston, Ont. K7L 3N6.

Previous research has shown that the nBOR is involved in the processing of visual information for compensatory head and eye movements. It has also been reported that these neurons have large receptive fields and respond best to large visual stimuli moving slowly in a particular direction. Two classes have been described, those preferring movement up, and those preferring movement down along the vertical axis. The present study confirms these findings, but describes several classes of neurons. Broadly tuned neurons have high spontaneous rates, but many narrowly tuned neurons have low spontaneous rates. Subfield stimulus presentation indicates that responses are not uniform across the receptive field, that is, there is an area of maximum excitation. Furthermore, the minimal area for a directional response and the area for a maximum response vary considerably. This research was supported by MRC grant #MA 7244 to B.J.F.

397.5

CELLS IN THE TURTLE'S BASAL OPTIC NUCLEUS ARE DIRECTION SELECTIVE OVER A BROAD VELOCITY RANGE. A. F. Rosenberg and M. Ariel. Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260

Retinal direction selective (DS) ganglion cells are thought to provide input to a neural circuit controlling optokinetic nystagmus through the BON of the accessory optic system. Since the BON is a small ventral brainstem structure lying directly above the cranial floor, in vivo recordings are difficult. In our experiments, we use a novel in vitro brain preparation (eyes attached, telencephalon removed) where the brain is inverted so that the ventral side is exposed and the BON is readily accessible. Stimulation is achieved by projecting a moving spot or random dot pattern onto the contralateral eyecup. The brain is continually perfused with oxygenated physiological media and is viable for up to 10 hours after decapitation.

Visually responsive cells were recorded extracellularly in the ventral brainstem, presumably in the BON. Using visual landmarks, recording of these BON cells was often possible in the first electrode penetration. We found that the BON contains almost exclusively DS cells. These cells lacked narrow velocity tuning in both the preferred and null directions. The direction selectivity of the cells was very pronounced for a wide range of illuminations; the ratio of preferred to null responses ranged from 4:1 to 25:1.

Supported by EY05978 and BRSG 2S07RR0784-21.

397.6

NEURONS OF THE MEDIAL TERMINAL ACCESSORY OPTIC NUCLEUS OF RAT ARE POORLY COLLATERALIZING. R.A. Giolli, R.J. Clarke , R.H.I. Blanks, Y. Torigoe & J.H. Fallon. Dept. Anatomy & Neurobiology, Coll. Med., Univ. Calif., Irvine, CA 92717.

Most neurons of rat medial terminal nucleus (MTN) project to the nucleus of the optic tract (NOT), but some project to other brainstem nuclei involved in contolling eye movements. The possibility that MTN-NOT neurons collateralize to innervate other MTN targets is studied with two retrograde flourescent tracers. Flourogold was injected into the NOT, and Fast blue was injected into one of the following nuclei: the ipsilateral supraoculomotor periaqueductal gray and the dorsal cap of the inferior olive and the contralateral visual tegmental relay zone, dorsolateral basal pontine nucleus and superior and lateral vestibular nuclei. The results show that nearly all MTN neurons are single-labeled in all paired injections of the NOT and each of these other nuclei. About 70% of MTN cells project to the NOT and 4-9% to each of the other nuclei. Only around 1% of MTN cells are double-labeled showing that the MTN-NOT neuron population is distinct from neuron pools projecting to the other outflow areas. These data reveal that virtually all MTN neurons are projection neurons. Supported by NSF grant BNS-8612919 and NIH grant NS-15321. RJC is a Brazilian National Research Council (CNPq) Scholar.

397.7

PROJECTIONS OF SUPRAGENICULATE PRETECTAL NUCLEUS IN RABBIT. K.M. Gregory[1], R.A. Giolli[2], R. H. I. Blanks[2], & Y. Torigoe[2], Dept. Anat. & Physiology, CSU Long Beach, CA. 90840[1], & Dept. Anat.& Neurobiology, CA. Coll. Med. U.C. Irvine, CA. 92717[2].

The suprageniculate pretectal nucleus (SGp) in the rabbit consists of large darkly stained cells located posterior to the nucleus of the optic tract (NOT). Injection of ^{3}H-leucine into the SGp of rabbits shows that it projects ipsilaterally to: str. gri. superficialis (SGS) of superior colliculus (SC), str. gri. profundum (SGP) of SC, olivary pretectal (PO), NOT, medial pretectal area, periaquaductal gray, thalamic suprageniculate, and lat. hypothalamus. The anterior pretectal nucleus was conspicuous by the absence of either fiber or terminal label. Contralateral projections are to SGS, PO, SGP and SGp. Injection of HRP into the SC confirms the SGp-SC bilateral connections of the SGp and also shows that the SC projects to the ipsilateral SGp. The only cortical label was in the temporal area and was probably due to leucine uptake in the medial geniculate. Thus the rabbit SGp is distinct from the thalamic suprageniculate nucleus which in the cat has projections to the insular cortex.
Supported by NSF Grant # BNS-8612919 to RAG & RHIB.

397.8

THE SPATIAL ORGANIZATION OF THE TELENCEPHALIC PROJECTION TO THE PRETECTAL LENTIFORM NUCLEUS OF THE MESENCEPHALON (LM) IN CHICKEN. Stefan R. Bodnarenko and Olivia C. McKenna. Biopsychology Program, Hunter College, N.Y., N.Y. 10021 and Biology Dept., City College, N.Y., N.Y. 10031.

The lentiform nucleus of the mesencephalon (LM) is a pretectal structure that receives its afferents, which we have shown to be spatially organized (JCN 269:3 '88), primarily from the retina. In this study we used the HRP retrograde tract tracing technique to determine whether a projection from the visual telencephalon to the LM in chickens is also spatially organized and to demonstrate the cells of origin. A single population of labeled neurons, with somata measuring 678.9 μm^2 on average, was identified after injections into the LM. These round or stellate-shaped neurons were confined to the lateralmost portion of the ipsilateral accessory hyperstriatum (HA), throughout its caudal to rostral extent, except for the rostralmost portion of HA. Partial injections into the LM revealed a spatial organization of the projection neurons. Labeled neurons in the caudal two-thirds and in more ventral portions of the projection area projected to the dorsal LM. Labeled neurons throughout the entire rostrocaudal extent of the projection area, but in more dorsal regions caudally, projected to ventral LM. Labeled neurons midway on the dorsoventral axis within the projection area projected to the middle LM.

These findings, suggest that a single population of projection neurons, which is confined to a specific portion of the HA, projects in a spatially organized manner to the LM. This study, in conjunction with electrophysiological evidence for a retinotopic organization of the HA and our recent report of the retinotopic organization of the LM, suggests that specific regions of LM receive a spatially organized projection from corresponding portions of the retina, either indirectly through the HA of the visual telencephalon or directly from the retina. The nucleus of the optic tract in mammals, which is considered homologous to the LM, also shows a spatial organization of its afferents arising from the visual cortex. (Supported by NIH EY 03613)

397.9

GABA-ERGIC INPUTS TO THE NUCLEUS ROTUNDUS IN PIGEONS (*Columba livia*). T. Shimizu, H. J. Karten, and W. Woodson*. Department of Neurosciences, M-008, School of Medicine, University of California, San Diego, La Jolla, California 92093.

The nucleus rotundus thalami (Rt) in birds plays an important role in visual information processing. Rt receives a non-topographic bilateral projection from layer 13 of the optic tectum, as well as from the subpretectalis complex (SP/IPS) and nucleus reticularis superior thalami (RS). Rt projects to the ectostriatum, a visual structure in the avian telencephalon (Benowitz and Karten, *J. Comp. Neurol.*, 167:503, 1976).

Antibodies directed against glutamic acid decarboxylase (GAD), the enzyme responsible for the synthesis of gamma-aminobutyric acid (GABA), labeled a major axonal plexus in Rt, but did not label any somata. Immunohistochemical and neuroanatomical tracing techniques were used to determine the source of input of this inhibitory neurotransmitter. GAD immunoreactivity was not found in somata of layer 13 of the tectum, but was present in somata of SP/IPS and RS. When the anterograde tracer phaseolus vulgaris leucoagglutinin (PHA-L) was injected into layer 13 of the tectum, dense PHA-L positive axons were found in Rt. These axons, however, did not express GAD immunoreactivity. When PHA-L was injected into SP/IPS, we found that some PHA-L positive axons in Rt were also GAD positive. Lesions of SP/IPS caused a significant decrease of GAD positive axons in Rt.

Thus, the source of GAD positive axons in Rt is at least partially from SP/IPS, not the optic tectum. Since SP/IPS itself is a target of the tectal efferents from layer 13, the SP/IPS-rotundal pathway may provide feed-forward inhibitory information to the tecto-rotundo-ectostriatum system.

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397.10

NUCLEUS ISTHMI IN THE PIGEON: AN HRP STUDY. L. Telford, Y-C. Wang* and B.J. Frost. Departments of Psychology and Physiology, Queen's University, Kingston, Canada, K7L 3N6.

Reciprocal connections between the nucleus isthmi (NI) and the optic tectum (OT) have been reported for a variety of mammalian and nonmammalian species. As this nucleus is divided into anatomically distinct parvocellular (Ipc) and magnocellular (Imc) divisions in the pigeon, we investigated tectal projections from each subdivision. Cell bodies in the deeper tectal layers were labelled after HRP was injected into Imc, suggesting that there is a direct connection between deep tectal layers and Imc. No labelled fibres were found in superficial cell layers. Long axons were also found coursing ventromedially from Imc. towards the brachium of the superior colliculus. After injection of HRP into superficial tectal layers, labelled cell bodies were found in Ipc. Projections were localized to a small group of cells within Ipc, suggesting that inputs are topographically organized within the nucleus, as reported by Hunt et al. (1977). This corresponds to our electrophysiological data (Wang and Frost 1987) which show that retinotopic mapping is maintained from tectum to NI.

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397.11

RETINOTOPOGRAPHIC ORGANIZATION OF OPTIC PROJECTIONS IN RANA PIPIENS. K. V. Fite and N. Montgomery, Neuroscience & Behavior Program, University of Massachusetts/Amherst

The retinotopic organization of the anuran visual system has been reinvestigated using anterograde and retrograde transport of HRP following small retinal lesions, as well as restricted HRP injections of tectum and pretectum.

The first nuclei to receive optic axons in the CNS are the preoptic and suprachiasmatic nuclei, innervated by the dorsal portion of the optic nerve containing axons from all retinal quadrants. Axons from each retinal quadrant enter the chiasm as bands of fibers and expand across the chiasm in the order—nasal, ventral, temporal and dorsal. Fibers destined for the optic tectum become more laterally placed, while those destined for thalamus and pretectum become more medially located. Two mesencephalic retinal projections, to the optic tectum and pretectal lentiform nucleus (nLM), are organized with the temporal quadrant located anteriorly, and the nasal quadrant located posteriorly. In the thalamus, 4 retinotopic maps, n. Bellonci, corpus geniculatum, rostral visual nucleus and posterior thalamic neuropil, are organized as mirror-image reversals of the tectal and pretectal maps. All retinal quadrants contribute to the basal optic nucleus; however, retinotopy could not be established for this nucleus, nor for the pretectal uncinate neuropil.

Such results are relevant for further understanding how retinal axons are organized prior to innervation.

397.12

MOTION SENSITIVITIES OF THE VISUAL CLAUSTRUM: INTERACTION OF SPEED AND DIRECTION. R.G. Carey and K.M. Horn. Div. Neurobiol., Barrow Neurol. Inst., Phoenix, AZ 85013 and Ctr. Vis. Sci., Univ. Rochester, Rochester, NY 14627.

The dorsal claustrum is a little known subcortical nucleus that has extensive connections with both cortical and thalamic visual areas. Our investigations of the visual characteristics of this nucleus in the ferret have shown that the vast majority of these neurons are sensitive to moving stimuli. The major features of moving stimuli are direction and speed, and many neurons within the ferret visual claustrum are sensitive to both of these features. Nearly 50% of our sample can be classified as either directional or directionally biased. These responses can be divided into two groups based upon the range of speeds where directionality is manifest. One-third of the cells demonstrate directionality across a broad range of speeds, with the average range extending from 50-1000 deg/sec. The remaining directional neurons show directionality within a narrow range of speeds. Further, the remaining neurons that were not directional when tested at the preferred speed did not show any directional preferences when tested at other speeds.

Claustral neurons respond to a broad range of speeds and appear suited for the detection of motion and not for the extraction of specific features concerning motion or stimulus form. These findings support the hypothesis of Boyapati and Henry (1985) that the claustrum is involved in motion detection. Also, those neurons that manifest directionality across a broad range of speeds may be involved in the detection of the direction of motion.

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397.13

NEURONS PROJECTING TO THE RETINA: AFFERENTS AND POSSIBLE FUNCTIONS. G. Marin, S. Bodnarenko, O. McKenna, and J. Wallman, Biology Dept., City College, City University of N.Y., New York., N.Y. 10031 and Biology Dept., Ramapo College of N.J., Mahwah, N.J. 07430.

The centrifugal fibers projecting to the avian retina originate in the isthmo-optic nucleus (ION). ION neural activity is strongly driven by a retinotopically organized visual input from the ipsilateral tectum, and, we find, is also modulated by saccadic eye movements. Since degeneration studies years ago suggested an input to the ION from the ocular motor nuclei, we reinvestigated the afferent connections to the ION using the HRP technique.

The ION of four-week-old chickens was injected using KCl-HRP-filled micropipettes, after localizing the nucleus by recording its visually evoked neuronal activity. Injections were small and restricted to the ION.

We found no trace of retrogradely transported HRP in any of the ocular motor nuclei. Only cells in the ipsilateral tectum were consistently labeled. The majority of the neurons had cell bodies located in lamina h of the stratum griseum et fibrosum superficialis. The long axis of their dendritic arborizations was perpendicular to the tectal laminae, with shorter dendrites extending upward, toward lamina g, and thicker and longer ramifying branches extending downward. Because no dendrites were observed above lamina g, where retinal fibers terminate, tecto-ION cells in the chicken, as in the quail, do not receive a direct projection from the retina. Instead, their larger dendrites directed toward a deeper layer suggests that the visual input to the tectum is integrated with other signals, including ones from the saccade-generating circuitry, to form the output to the ION. Experiments addressing this possibility will be presented.

397.14

SUBCORTICAL CONNECTIONS OF INFERIOR TEMPORAL CORTEX OF OWL MONKEYS. R. E. Weller and J. H. Kaas. Dept. of Psychology, Univ. of Alabama at Birmingham, AL 35294 and Dept. of Psychology, Vanderbilt Univ., Nashville, TN 37240.

Previously we described the connections in owl monkeys of subdivisions of inferior temporal cortex (IT) with the pulvinar complex. We now describe the remaining subcortical connections of IT. Injections of ^{3}H-proline were made in caudal (IT_C) or rostral (IT_R) subdivisions of IT of eleven owl monkeys (Aotus trivirgatus). Projections found included those to the amygdala, claustrum, caudate nucleus, putamen, intralaminar nuclei, pretectal nuclei, pons, and, as described previously, subdivisions of the pulvinar complex. Many of these structures also receive input from other subdivisions of extrastriate visual cortex (Graham, J., Lin, C.-S., and Kaas, J. H., J. Comp. Neurol., 187:557, 1979). The input to the amygdala, however, was not previously seen, and distinguishes the subcortical connections of IT from those of more caudal subdivisions of visual cortex. Finally, there were differences between the connections of rostral versus caudal IT. As an example, IT_R had much stronger connections with the amygdala than did IT_C. Differences such as this one support the proposed distinction in owl monkeys between the IT_R and IT_C subdivisions (Weller, R. E. and Kaas, J. H., J. Comp. Neurol., 256:137, 1987). Supported by NIH Grants EY-07147 and EY-02686.

397.15

SOME RETINOFUGAL AXONS IN ADULT FERRETS INCREASE IN CALIBER AS THEY PASS FROM EYE TO BRAIN G.E.Baker* and R.W.Guillery University of Oxford, Dept. of Human Anatomy, Oxford, U.K.

During a study of fiber order in the ferret retinofugal pathway we have noticed that the diameters of the largest axons in the optic tract appear greater than those in the intraorbital optic nerve. We have quantified this and now show that in the optic tract there are axons with diameters greater than the largest found in the post-optic segment of nerve.

Vibratome sections (200μm) from the intraorbital nerve and optic tract were osmicated and embedded in Epon. All sections were treated identically. Semi-thin sections from nerve and tract were stained with phenylene-diamine and internal diameters of axons greater than 4μm (judged by comparison with standard circles) were drawn at x1600 and then measured using the Bioquant image analysis system.

Counting the number of fibers with inside diameter greater than 5μ (D = perimeter/π) gave the following results: Ferret 1: intraorbital nerve = 304 fibers, tract = 989 fibers. Ferret 2: intraorbital nerve = 83 fibers, tract = 462 fibers.

These large fibers in the tract must be retinofugal since 3-4 months after a monocular enucleation all the largest fibers in the contralateral tract degenerate. It remains to be determined whether these fibers taper gradually or change diameter over a short distance as they pass from one glial environment to another.

(Supported by MRC grant PG8324037)

397.16

THE RELATIONSHIP BETWEEN THE PROJECTIONS FROM VISUOCORTICAL AREAS V1 AND V2 TO THE THALAMIC RETICULAR NUCLEUS IN THE RABBIT. **J.W. Crabtree*. (SPON: M. Stryker). Dept. of Human Anatomy, Univ. of Oxford, U.K.**

The traditional view of the thalamic reticular nucleus (TRN) is that adjacent areas of the overlying neocortex map onto adjacent areas within the plane of the TRN. A previous study (Crabtree, J.W. and Killackey, H.P., Neurosci., Suppl., 22: 804, 1987) showed that, in the visual sector of the rabbit's TRN, focal areas of visuocortical area V1 are represented by "slabs" that lie parallel to the borders of the nucleus in the rostrocaudal dimension. In the present study of the rabbit, injections of HRP or [^{3}H]proline were made into visuocortical area V2. The resultant anterograde labelling in thalamus was analyzed and compared with that following tracer injections into V1.

A single injection in V2 results in a single focus of label that is located in the dorsocaudal, visual sector of the TRN. Injections restricted to V1 produce slabs of label that are confined to the outer two-thirds of this sector, while those involving V2 result in slabs of label within the inner one-third of the sector. Only the outer aspect of the TRN's visual sector projects to the dLGN, as revealed by retrograde labelling in the TRN following injections of HRP in the dLGN.

These findings in the rabbit show that, contrary to the traditional view, the adjacent cortical areas V1 and V2 do not map onto adjacent TRN areas along the plane of the nucleus, but rather they map onto adjacent areas orthogonal to the plane of the nucleus. Thus, the neocortex is represented in a discontinuous fashion along the plane of the TRN. The target of the efferents from the inner aspect of the TRN's visual sector, which receives the V2 projection, remains to be defined. (Supported by the MRC, Grant No. 8324037)

397.17

ULTRASTRUCTURAL EXAMINATION OF TECTAL TERMINATIONS IN THE VENTRAL LATERAL GENICULATE NUCLEUS (LGN_V) IN THE CAT. M.D. Noseworthy* and G.D. Partlow, Department of Biomedical Sciences, University of Guelph, Guelph, Ont., N1G 2W1.

To further understand the visual process, the functional morphology of the ventral lateral geniculate nucleus (LGN_V) was investigated. Tectal input was examined using degenerative tracing methods. Unilateral ablation of the superior colliculus was performed in 7 cats with care to avoid damage to the overlying visual cortex. Following survival times of 2-12 days, euthanasia was performed, and the tissue prepared for electron microscopic examination. Thin sections were cut from the ventromedial region, described as being that area receiving tectal efferents (Graham, J., J. Comp. Neurol., 173:629-654, 1977). Electron micrographs were examined for synaptic organization.

Degenerating boutons were localized in this region. Qualitatively, they were most often axodendritic, type F connections. Type F refers to boutons containing flattened vesicles, which corresponds to an inhibitory synapse. Preliminary results would indicate that normal synaptic connections were most frequently of this classification. This conveys the possibility of an inhibitory role by the tectal efferents to the LGN_V. With combined visuomotor-vestibular function, such inhibition is yet to be functionally quantified.

397.18

FUNCTIONAL ORGANIZATION OF THE VENTRAL THALAMUS IN THE TREE SHREW. M. Conley, B. Friederich-Ecsy* and I.T. Diamond. Dept. Psychology, Duke Univ., Durham, NC 27706.

The ontogenetic and comparative cytoarchitectonic studies of Rose ('42) led him to conclude that the ventral thalamus consists of the ventral lateral geniculate nucleus (GLv) and the thalamic reticular nucleus (TRN), and that reticular portions of GLv were often confused with the TRN. In this report we have used transport and immunocytochemical methods to study the functional organization of the GLv in the tree shrew and have identified 5 subdivisions, each with specific connections, supporting Rose's view: the external segment and intergeniculate leaflet are retino- and tecto-recipient and contain numerous ChAT-immunoreactive fibers and terminals; the internal segment projects to the tectum and anterior pretectal nucleus and, with the external segment, receives projections from layer V of striate cortex. Two large medial subdivisions share a common border with the TRN and are divided into rostral compact and caudal reticular segments by a ChAT-immunoreactive fiber band. Both subdivisions are rich in calmodulin-kinase but poor in GABA immunoreactivity--the reverse of TRN. The rostral segment has reciprocal connections with the nucleus of the optic tract; the caudal segment receives projections from the cerebellum and projects to the nucleus reticularis tegmenti pontis. Supported by NIMH MH04849, NIH NS19206, NSF BNS8607779 and "Deutsche Forschungsgemeinschaft".

397.19

THE VENTRAL LATERAL GENICULATE IN A LIZARD.
Neil M. Montgomery & Robin Mergendalh*
University of Massachusetts, Amherst, Mass.01003

The term ventral lateral geniculate (VGL) is used extensivily to describe a visual nucleus in the ventral thalamus of a number of vertebrates yet the relationship between these structures is unknown. In the present study the connectivity of VGL in Anolis carolinesis has been investigated using HRP.

In lizards the VGL is a large crecent shaped structure consisting of a medial cell plate and lateral neuropil. The neurons of the cell plate have palisade-like dendritic fields while the neuropil contains stellate neurons. The neuropil receives retinal afferents while the cell plate receives tectal afferents. Further, the tectal map in VGL is topographically organized and is a 'mirror-image' of that found in the tectal lobe.

Two efferent pathways arise from the VGL cell plate. The dorsal bundle projects to the pretectum reaching the medial pretectal nucleus and the nucleus of the tectal gray. The ventral pathway projects to the accessory optic nucleus, the red nucleus and premotor cell groups lateral to the oculomotor nuclei. The relationship between these projections and those found in other vertebrates will be discussed.

CEREBRAL ISCHEMIA IV

398.1

ISCHEMIA PRODUCES A LOSS OF SOMATOSTATIN IMMUNOREACTIVITY IN ALL HIPPOCAMPAL SUBFIELDS. B. Fadeel*, J. Franck, D. Kunkel and P. Schwartzkroin. Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

The CA1 and CA3 subfields of hippocampus are often necrotic following disease processes, particularly temporal lobe epilepsy. We have been interested in the effects such selective cell loss has on the physiology and restructuring of remaining hippocampal neurons and in the relevance these processes may have for the clinical population with similar damage.

Selective loss of CA1 is produced experimentally, and has been observed in humans, following transient ischemia. We used this approach, produced in adult male Wistar rats by 15 minutes of carotid artery occlusion with vertebral artery cautery, to examine the consequences of CA1 loss on remaining dentate and hippocampal neurons. We have observed that ischemia results in near-complete loss of the somatostatin immunoreactivity normally present in interneurons throughout the hippocampus and dentate gyrus. This loss was observed up to 6 weeks following ischemia and appeared to be specific in that GAD immunoreactive neurons were still apparent. Studies are currently ongoing to determine if reduced somatostatin represents selective cell death or decreased peptide expression.

Somatostatin has been proposed to play a critical role in modulating inhibitory interneuron function. Selective loss of this population of cells or of the peptide they contain, particularly in regions where principal cells remain intact, may alter the excitability of remaining hippocampus.

Supported by NIH-NINCDS grants NS20482 and NS25155.

398.2

DELAYED DECREASE IN BRAIN MITOCHONDRIAL CYTOCHROME aa_3 CONCENTRATIONS FOLLOWING ANOXIA. K.R. Wagner, M. Kleinholz* and R.E. Myers. Research Service., VA Medical Center, Cincinnati, Ohio 45220.

Hyperglycemic cats exposed to 8 minutes of anoxia develop a delayed appearance of neurologic signs including fasciculations and focal seizures involving muscles innervated by cranial nerves. These "symptomatic" cats show elevations of brain lactate and decreases in phosphocreatine due to impairments in brain mitochondrial respiration. Impairment in mitochondrial respiration appears to be due to a decrease in electron transport chain function as demonstrated by a 50% reduction in uncoupler-stimulated respiration and a 57% decrease in cytochrome oxidase activity. In contrast, these biochemical alterations are not present following anoxia in similarly exposed normoglycemic cats or in hyperglycemic cats prior to development of neurologic signs.

In the present study, only brain mitochondria isolated during the postexposure period from cats with neurologic signs showed a significant decrease (45%) in cytochrome aa_3 concentrations. Cytochrome b levels were similar in all experimental groups. Thus, these results suggest that the impairment in cytochrome c oxidase activity in postanoxic symptomatic cats may be due to a loss of heme aa_3. (Supported by Veterans Administration Medical Research Service funds).

398.3

ROLE OF LACTIC ACID TRANSPORT IN THE CNS. W. Walz. Dept. of Physiology, Coll. of Med., Univ. of Saskatchewan, Saskatoon, S7N 0W0, Canada.

Lactate/lactic acid is thought to be a mediator of irreversible brain damage during anoxia and ischemia. Lactate release of cultured astrocytes and of cultured neurons was investigated. The internal lactate concentration remained stable at 23 mM in both cell types. A lactate/proton cotransport mechanism was found to operate in both cell types. They therefore will excrete lactic acid into the ECS. The release rate of lactic acid is about 2000 in astrocytes and 300 nmol xmg^{-1} x hr^{-1} in neurons. Block of respiration increased the neuronal rate to match glial release, confirming that astrocytes have a higher anaerobic glycolysis rate than neurons. High glucose (30 mM) increased lactic acid release only in astrocytes. K^+-induced cytotoxic glial swelling doubled the release rate, but only after swelling was accomplished. Content did not change, confirming that lactate is not involved in brain cell swelling. The action of lactate/lactic acid on neuronal transmission in the hippocampus was investigated. Lactate (30 mM) did not lead to irreversible damage. Lactic acid, however, due to a decrease in pH, damaged synaptic transmission irreversibly. Thus, lactate as an anion can be very well tolerated by the CNS. However, lactic acid will damage synaptic transmission.

The author was an MRC Scholar and is presently an MRC Scientist.

398.4

ELECTRICAL, ION TRANSPORT AND METABOLIC CHANGES DURING BRAIN ISCHEMIA IN RAT: COMPENSATORY BUT NOT THRESHOLD EVENTS. C.N. Raffin*, M. Harrison*, T.J. Sick and M. Rosenthal. *Dept of Neurology, University of Miami Medical School, Miami, Fl 33101*

Ligation of the carotid arteries after electrocoagulation of the vertebral arteries (the 4-vessel occlusion model of Pulsinelli and Brierley) produces an event sequence in rat brain that includes EEG suppression, anoxic depolarization (AD) with maximal increases in extracellular potassium ion activity (K^+o), and maximal decreases in tissue oxygenation (tPO_2) with reduction of the mitochondrial electron transport carriers. To determine whether these changes are predictive or determinate of subsequent events, and to provide insights into mechanisms of EEG suppression and AD, electrode and optical techniques were employed in rats anesthetized with pentobarbital. No threshold levels of cytochrome a,a_3 reduction, tPO_2 or K^+o could be determined for EEG suppression. Also, no threshold values of tPO_2, reduction of cytochrome a,a_3 or K^+o could be found that were predictive of AD onset. However, latency to EEG suppression was inversely correlated with latency to AD, to maximal decreases in tPO_2 and to maximal reduction of cytochrome a,a_3. In contrast, latency to AD was proportional to latency of subsequent maximal decreases in tPO_2 and cytochrome a,a_3 reduction. These data do not support the concept that EEG suppression and AD are each produced by thresholds derangements of mitochondrial function. On the contrary, they suggest that EEG suppression is a compensatory process to spare oxgyen for the production of energy for other activities such as ion transport and to avoid the consequences of the loss of ion homeostasis. The early suppression of EEG also suggests the activity of a sensor of potential energy failure, the identity of which, in ischemia, remains unknown.

398.5

CEREBRAL FOCAL ISCHEMIA ALTERS LEVELS OF MOLECULAR FORMS OF ACETYLCHOLINESTERASE (AChE). S.P. Mahadik, A. Korenovsky*, C.G. Wakade, & S.E. Karpiak (SPON: H. Laev). Div. Neuroscience, NYS Psychiatric Inst., Depts. of Psychiatry, and Biochemistry & Molecular Biophysics, Physicians & Surgeons, Columbia U., New York.

Physostigmine treatment after focal cerebral ischemia in rat was shown to increase cerebral blood flow and reverse ischemic injury. To further investigate the molecular mechanism of cholinergic dysfunction in ischemia we report here changes in levels of AChE and its molecular forms (10S, mostly membrane and 4S, mostly soluble) in the primary (parietal) and peri-infarct (frontal, occipital & temporal) areas after focal ischemia in rat. Cortical focal ischemia was produced by permanent middle cerebral & common carotid artery occlusion [MCAo+CCAo] with 1 hr temporary contralateral CCAo. AChE was determined by Ellman's assay in presence of ISO-OMPA. Compared with the contralateral side, AChE levels were reduced by 25% in the primary infacrt area, and minimally in the peri-infarct areas. Analysis of molecular forms showed the 10S form was reduced by 41% and 4S form was increased by 84% in the primary area. Results indicate that during focal ischemia membrane AChE is released and converted to 4S, probably reflecting an alteration in membrane structure, contributing to a loss of cholinergic function. Since gangliosides protect cholinergic neurons in CNS injury, we are studying their effects on cholinergic enzymes.

398.6

EVIDENCE FOR *IN VIVO* RELEASE OF N-ACETYLASPARTYLGLUTAMATE DURING CEREBRAL ISCHEMIA IN THE RAT. J.K. Deshpande, G. Tsai, R.J. Traystman* and J.T. Coyle. Depts. of Anesthesiology and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Cerebral ischemic damage exhibits a predilection for certain brain regions such as hippocampus and may be mediated by activation of glutamatergic receptors. N-acetylaspartylglutamate (NAAG) is a neuropeptide localized to some glutamatergic pathways and may act as a neurotransmitter/modulator in hippocampus that activates NMDA receptors. To determine whether NAAG is released during transient cerebral ischemia, microdialysis probes were stereotaxically placed into hippocampal subfield CA1 and perfused with artificial CSF. Ischemia was induced in 5 rats by hypotension and carotid clamping (2 v-o model) for 30 min. Ten minute fractions of dialysate were collected prior to (-60, -40 and -10 min) and during ischemia (10, 20 and 30 min). NAAG levels were measured by a sensitive and specific radioimmunoassay (RIA). The spontaneous release of NAAG-like immunoreactivity (NAAG-LIR) was high (16.8 $\pm$ 2.3 pm/fraction) immediately after placement of the probe but declined to a stable level (3.6 $\pm$ 1.4 pm/fraction) after 60 min of perfusion. Ischemia resulted in a 4 to 6-fold increase in NAAG-LIR over preischemic levels. The results demonstrate that the RIA method is sufficiently sensitive to measure endogenous NAAG in dialysate. The NAAG release during ischemia suggests that NAAG may play a role in mediating ischemic neuronal injury.

398.7

DECREASED HIPPOCAMPAL NOREPINEPHRINE AND IMPAIRED SYNTHESIS FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA IN THE MONGOLIAN GERBIL. K.E. Moore and J.N. Davis, Depts. of Medicine (Neurology) and Pharmacology, V.A. and Duke Univ. Medical Centers, Durham, NC.

Hippocampal CA_1 pyramidal cells are selectively vulnerable to transient ischemic injury. We measured the level and rate of NE synthesis after 5 min. ischemia. Hippocampal NE fell from 0.29 ±.04 to 0.14 ±.05 ng/mg ($p<.05$) at 1 hr. following ischemia, compared to 0.21 ±.04 at 1 hr. in sham operated controls. At 24 and 48 hrs. post-surgery, NE levels had returned to baseline. To determine why NE was decreased after ischemia, the DOPA decarboxylase inhibitor NSD-1015 was used to estimate NE synthesis. DOPA is barely detectable in the normal brain, but 30 min. after NSD-1015 administration 13 ±1 pg/mg accumulates. DOPA concentrations were 19 ±2 in ischemic vs. 29 ±4 pg/mg in sham operated gerbils 1 hr. post-surgery ($p<.05$), 23 ±5 vs. 38 ±13 pg/mg at 6 hrs., and 10 ±1 vs. 17 ±6 pg/mg at 12 hrs. These data indicate that 1) after surgery there is a transient decrease in hippocampal NE; 2) NE synthesis increases in ischemic and sham operated gerbils; and 3) ischemic gerbils do not increase NE synthesis as much as sham operated controls. If ischemia causes NE release, impaired NE synthesis would result in a net loss of NE. The loss of NE may aggravate neuronal loss after transient ischemia. We speculate that transient ischemia impairs phosphorylation of tyrosine hydroxylase in noradrenergic neurons.

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398.8

NOREPINEPHRINE (NE) AND SEROTONIN (5-HT) RELEASE IN THE DORSAL HIPPOCAMPUS DURING GLOBAL ISCHEMIA. L. A. Phebus*, J. A. Clemens and R. Mincy* (SPON: J. A. DiMicco). The Lilly Research Labs, Indianapolis, IN 46285.

Male Wistar rats were anesthetized, both vertebral arteries were cauterized closed and reversible clamps were loosely placed around both common carotid arteries. At the same time, a miniature dialysis probe was permanently implanted in the CA-1 area of the dorsal hippocampus. The next day, the output of the dialysis probe was directly connected to an automated microbore HPLC which assayed the brain dialysate samples of NE, 5-HT, 5-HIAA, DOPAC and HVA at fifteen minute intervals. After a baseline was established, ischemia was induced by tightening the clips around the carotids. Following thirty minutes of ischemia, the carotid clips were released and reperfusion begun. In a second experiment, after a baseline was established, CSF containing 10^{-3}M ouabain was perfused through the probe and its effects on neurotransmitter release were measured.

During ischemia there was a large increase in extracellular NE and 5-HT and a decrease in 5-HIAA, DOPAC and HVA. On reperfusion, the levels of these neurochemicals returned to near normal. Ouabain administration via the dialysis probe also released NE and 5-HT into the extracellular fluid. The release of these transmitters during cerebral ischemia may play a role in the damage produced by this insult.

398.9

EFFECTS OF DIPYRIDAMOLE AND SOLUFLAZINE, NUCLEOSIDE TRANSPORT INHIBITORS, ON THE RELEASE OF PURINES FROM RAT CEREBRAL CORTEX. M.H. O'Regan*, J.W. Phillis and G.A. Walter*. Dept. of Physiology, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Manipulation of adenosine transport as a means of enhancing adenosinergic tone has been possible using selective transport inhibitors. These agents can depress neuronal activity, induce sleep in dogs, depress locomotor activity in mice, exert anticonvulsant effects and enhance hypoxia-evoked cerebral vascular reactive hyperemia. The cortical cup technique was used to determine the effects of dipyridamole and soluflazine on cortical perfusate levels of adenosine and its metabolites, measured by HPLC, before, during and after a 5% oxygen challenge. Hypoxia-evoked adenosine, inosine, hypoxanthine and xanthine levels were suppressed following soluflazine (50 µg/kg iv), consistent with inhibition of a bidirectional transporter. In contrast, dipyridamole (0.5 mg/kg iv), which purportedly does not cross the blood-brain barrier, potentiated hypoxia-induced releases of adenosine in comparison with the saline injected controls, while hypoxanthine and xanthine levels declined. These results illustrate a potential complication in the use of these inhibitors, since the bidirectional nature of facilitated nucleoside transport in brain allows for either symetrical or assymetrical inhibitory effects leading variously to increased or decreased levels of endogenous adenosine in the interstitium.

398.10

DECREASED EXCITATORY AMINO ACID RECEPTORS IN GERBIL CAUDATE FOLLOWING 10 MIN FOREBRAIN ISCHEMIA. L.P. Miller* and T.R. Insel (SPON: J. Johannessen). Vet Adm Med Ctr, Wash D.C. 20422, Dept. Pharm., Georgetown Univ. Sch. Med., Wash D.C. 20007 & Lab. Clin. Sci., NIMH, Poolesville, Md. 20837.

Subjecting rats or gerbils to short-term (5-10 min) forebrain ischemia initiates a series of events which progresses over a period of 2-4 days to cell death in a number of brain regions. There is accumulating support for excitatory amino acid (EAA) mediated neurotransmission in hippocampal cell death including: (1) presence of a high density of EAA receptors (2). inhibitors of EAA-mediated neurotransmission attenuate the observed cell death. There is also support for EAA involvement in cell loss in the caudate, since lesioning of the cortical projection to the caudate (glutamatergic fibers) will attenuate cell death in this region following forebrain ischemia. In the present study the extent of alteration in binding to glutamate receptor subtypes in the caudate was measured post-ischemia. Ischemia in 10-12 week old female gerbil was induced by bilateral clamping of the common carotids for 10 min under 2.5% halothane anesthesia. At 7 days post-ischemia the animals were sacrificed by decapitation, brains removed and frozen. EAA receptors were characterized by N-methyl-D-aspartate (NMDA) displaceable 3H-glutamate binding to the NMDA receptor, 3H-AMPA binding to the quisqualate receptor and 3H-Kainic acid binding to the KA receptor.

	Region	Percent of Control		
		NMDA	AMPA	KA
(*sig,p<0.05)	lateral caudate	79*	86	88*
	medial caudate	84	94	94

Our results (1) show that binding to EAA receptors in the lateral but not medial caudate is altered following ischemia (2) are consistent with EAA receptor involvement in cell death observed in this region of the basal ganglia.

398.11

INHIBITION OF PYRUVATE DEHYDROGENASE FOLLOWING ISCHEMIA. F.A.Welsh and Y.Katayama*. Division of Neurosurgery, Hospital of Univ. of Pennsylvania, Philadelphia, PA 19104.

Pyruvate dehydrogenase (PDH) is a rate-controlling step in energy metabolism. Thus, loss of PDH activity following cerebral ischemia may impair the generation of high-energy phosphates. In the present experiments, cerebral ischemia was produced in pentobarbital-anesthetized gerbils, using bilateral carotid artery occlusion. Gerbils were pretreated with 20 mmol/kg glucose to induce hyperglycemia. After 20 min ischemia, the brain was reperfused for intervals up to 4 hr. PDH activity in unanesthetized, normoglycemic gerbils was 50 ± 3 mmol/kg/hr in cortex and 50 ± 8 in caudate. In anesthetized, hyperglycemic animals, PDH activity was 18 ±4 and 18 ± 3 mmol/kg/hr in cortex and caudate, respectively. Ischemia increased PDH activity to 38 ± 13 in cortex and to 39 ± 5 mmol/kg/hr in caudate. However, after 20 min reperfusion, PDH activity decreased markedly to 7 ± 3 in cortex and 5 ± 2 mmol/kg/hr in caudate. Over the next 4 hr of reperfusion, PDH activity recovered gradually to 15 ± 5 and 17 ± 5 in cortex and caudate, respectively. Tissue levels of phosphocreatine exhibited a secondary decrease in caudate, but not in cortex at 4 hr. These results indicate that PDH activity is diminished following cerebral ischemia and thus may cause a secondary failure of energy metabolism. Further, the decrease PDH activity in hyperglycemic animals may explain the known detrimental effects of hyperglycemia on ischemic brain injury.

398.12

ISCHEMIC EFFECTS ON NORADRENERGIC AND ENERGY METABOLITES IN THE CEREBRAL CORTEX OF YOUNG AND ADULT GERBILS. K. Kumami, B.B. Mrsulja, Y. Ueki, B. Djuricic and M. Spatz. Lab. of Neuropathology & Neuroanatomical Sciences, Nat. Institutes of Health, Bethesda, MD 20892

Relationship between ischemic changes in the cerebral cortical content of energy and noradrenergic metabolites was evaluated in young and adult gerbils. Groups of 3 weeks and 3 months old gerbils were subjected to 5 or 15 min of bilateral carotid artery occlusion alone or with 1 hour release. Animals were killed by microwave irradiation. Energy and monoamine metabolites were determined in the same sample of tissue. Ischemia (5 and 15 min) depleted energy metabolites but did not affect the content of either norepinephrine (NE) or homovanillic acid (HVA) in young and adults. At 1 hour of reflow after 5 and 15 min of ischemia, the levels of NE significantly decreased while those of HVA increased in adult but not in young. At this time a complete recovery of energy reserves was seen in young and adults. These results indicate that the ischemic change in homeostasis of energy metabolism is not directly associated with that of noradrenergic system in young and adult cerebral cortex. The observed disturbance in the metabolic pathway of NE during recovery period in adult but not in young animals suggests that the increased turnover rate (= release) reported in cerebral ischemia of adults may differ from that of the young brain subjected to ischemia.

398.13

THE RELATIONSHIP BETWEEN EEG AND BRAIN HIGH ENERGY METABOLITES DURING HYPOXIA. D.S. Smith, M.D., H. Miyake*, S. Nioka*, A. Zaman*, B. Chance*. Depts. of Anesthesia, Biochemistry/Biophysics, Univ.of PA., Philadelphia,PA 19104.

The relationship between brain energy and EEG was studied using 31-P Magnetic resonance spectroscopy during a gradually progressive hypoxic insult.Prior to the hypoxia, the animals were exchange transfused to hemoglobin (Hb) concentrations of 0.5-1.5 g/dl with a fluorocarbon oxygen carrier. Cats were anesthetized with ketamine, tracheotomized and mechanically ventilated, catheters were inserted in the femoral arteries and veins, and the skin and muscle of the skull were reflected. Brass screws were used for recording the EEG. Phosphorus spectra were obtained with a 2 cm coil placed on top of the skull, a 26 cm, 1.9 tesla magnet and a 90° pulse at 4 sec intervals. After the exchange transfusion, FiO_2 was lowered by increments until death occurred. Each level of FiO_2 was maintained for 12 min. The results demonstrated a distinct relationship between the change in EEG amplitude and brain levels of PCr. There was a threshold response below which EEG decreased as a linear function of PCr change. There was an increase in inorganic phosphorus, however, there was minimal change in ATP. The data demonstrates the close relationship between EEG power and level of brain energy metabolites with the strongest relationship for PCr and little change in ATP. The data provides further evidence that changes in the rate of ATP consumption or production are buffered by the conversion of PCr to ATP via creatine kinase.

CEREBRAL METABOLISM AND BLOOD FLOW III

399.1

PHASE CONTRAST MR IMAGING IN CEREBRAL ISCHEMIA AT 4.7 T. M.J. Quast*, G Ward*, R. Treaff*, T.A. Kent*. (sponsor: R.R. McKendall) Depts. of Neurology, Neurosurgery, and Marine Biomedical Institute, UTMB, Galveston, Texas.

In magnetic resonance imaging (MRI) and spectroscopic studies of the brain in the immediate post-arterial occlusion period, we noticed that heavily T_2-weighted images did not demonstrate a lesion for several hours while a perfusion deficit could be clearly seen (Kent, et al, AJNR, in press) and lactate and high energy phosphates were altered within a few minutes (Bradley, et al, AANS, 1988). We therefore have pursued other methods to image early changes. Studies indicate that fatty acid levels are increased in ischemia (Rehncrona, et al, J. Neurochem, 1982). Phase contrast imaging (Dixon, Radiology, 1984) provides a method to separate fat and water signals. We optimized the 180 refocussing pulse time shift so that maximum phase contrast occurred from signals originating at 1.3 ppm in the proton NMR spectrum. Difference images, obtained by subtracting each pixel from this and a spin echo image (TE 40, TR 1000) clearly demonstrated lesions in rats: 1) Before and after death by anesthetic overdose, in which increases in signal were observed to accumulate our time, first in cortical regions, and 2) focal ischemia by MCA occlusion, 3 cerebral vessel and single carotid artery occlusion, when lesions occurred prior to becoming visible on standard MRI. The mechanism of these changes is under investigation.

399.2

VALIDATION OF LASER-DOPPLER VELOCIMETRY IN THE CNS Lindsberg P J*, O'Neill J T*, Paakkari I*, Hallenbeck J*, Feuerstein G. (SPON:D Malcolm). Depts. of Neurology and Pediatrics, USUHS, Bethesda, MD 20814

Laser-Doppler velocimetry (LDV) is a new non-invasive method for continuous on-line monitoring of microvascular blood flow. LDV has previously been validated with more established methods in various other tissues, yet its validity and accuracy in the CNS has not been shown. We have compared LDV with the microsphere method (MS) using two independent laser probes placed dorsally on exposed lumbar (L5 laminectomy) spinal cord of anesthetized rabbits (n=7). Following baseline flow measurements spinal cord blood flow (SCBF) was increased (up to 50%) with iv infusion of phenylephrine (20-60 µg/kg/min) and decreased (up to 50%) with a bolus of chlorisondamine (10 mg/kg). The percentage changes of SCBF from the baseline obtained by LDV and MS agreed excellently ($r=.94$, $p<.0001$). The corresponding changes in tissue vascular resistance (VR) also correlated well ($r=.90$, $p<.0001$). On the contrary, there was less correlation ($r=0.38$) between the absolute SCBF values obtained by the two methods during baseline or altered SCBF. In conclusion, the validity of LDV in estimating the instantaneous SCBF (ml/100g/min) remains unproven, but is confirmed in monitoring temporal changes of SCBF and VR. Therefore, LDV proves a valuable tool for research manipulating CNS microcirculation by pharmacological or pathophysiological interventions.

399.3

ASTROGLIAL K^+ HOMEOSTASIS & LOCAL CEREBRAL BLOOD FLOW REGULATION R.P. Kraig & C. Iadecola. Department of Neurology, Cornell University Medical College, New York, N.Y. 10021

Neuronal activity is a major factor regulating local cerebral blood flow (lCBF). Increased interstitial concentration of K^+ ($[K^+]_o$) from neuronal activation was thought to influence lCBF by producing cerebrovasodilation. Recently, Paulson & Newman (Science 237:896, '87) speculated that astroglial $[K^+]$ changes may mediate the increase in lCBF. We sought to test this hypothesis by measuring changes in $[K^+]_o$ and lCBF elicited by local surface electrical stimulation (lSES) before & after reduction by Ba2+ of the rise in astroglial $[K^+]$ elicited from lSES (Canad J Physiol/Pharm 65:1038, '87). $[K^+]_o$ was measured with ion-selective microelectrodes (layers I & II, parietal cortex) and lCBF with a surface laser doppler probe in halothane anesthetized and ventilated rats (n=4). Before Ba^{2+} superfusion, lSES (10 Hz, 30 s) increased $[K^+]_o$ from 2.9 ± 0.1 (n=25) to 6.6 ± 0.3 mM (n=23) and lCBF to 173 ± 13% of control. Spreading depression (SD) increased $[K^+]_o$ to 40.8 ± 4.2 (n=5) and lCBF to 195 ± 12% of control. With Ba2+, resting $[K^+]_o$ fell to 2.3 ± 0.1 mM (n=14) but rose to 7.6 ± 0.6 (n=10) during lSES and 41.7 ± 3.0 (n=6) during SD while lCBF rose to 179 ± 9 and 180 ± 16% of control, respectively. Thus, lCBF changes are not directly correlated (duration & magnitude) to a rise in $[K^+]_o$ or (presumably) astroglial [K+]. Hence, the "coupling" between functional activity & lCBF is not based solely on changes in interstitial or astroglial $[K^+]$.

399.4

CORTICAL BLOOD FLOW DURING SEIZURES IN RATS WITH OPEN VERSUS INTACT SKULLS. E.M. Slaven*, J.C. Magee*, R.M. Zweifler*, and N.R. Kreisman. (SPON: F. Domer) Dept. of Physiol., Tulane Univ. Sch. Med., New Orleans, La 70112.

Cortical blood flow (CBF) was measured during serial seizures in rats with open or intact skulls to rule out the possibility that attenuation of ictal increases in CBF results from hemodynamic changes in the open cranium. Seizures were induced at 5-10 min intervals with pentylenetetrazol in anesthetized, paralyzed rats, and CBF was measured bilaterally by H_2 washout. Mean control CBF was faster in the right cortex and with the skull open. However, CBF rose 330-350% over control (n=20) during seizures early in the series and diminished to 120-150% over control during later seizures, regardless of whether the skull was open or intact or whether measurements were made in the right or left cortices. Although exposing the cortex results in overestimation of CBF values with the H_2 washout method, probably due to diffusion of H_2 into the atmosphere, it does not alter the percent increases in CBF measured during serial seizures. (Supported by NIH grant NS-17443)

399.5

MODIFICATION OF CEREBRAL HYPOMETABOLISM AFTER CORTICAL CONTUSION: COMPARISON OF GLUCOSE UTILIZATION RATES AND CYTOCHROME OXIDASE HISTOCHEMISTRY. M.J. Chen, T.M. Rolland*, S.A. Queen, and D.M. Feeney. Depts. of Psychol. and Physiol., UNM, Albuquerque, NM 87131.

A widespread reduction in cerebral oxidative metabolism, demonstrated by cytochrome oxidase histochemistry (CYOH) has been reported at 48 hr following right sensorimotor cortex ablation in rats (1). Amphetamine (AMPH, 2mg/kg; 24 hr post-contusion compared to vehicle controls; 24 hr pre-CYOH) significantly reversed this depression of CYOH staining in some brain areas remote from the injury. This parametric study further examined effects of right sensorimotor cortex contusion (SCC) and AMPH using both autoradiographic 2-Deoxy-D-Glucose (2-DG) and CYOH procedures. At 24 hr post-SCC or sham surgery, rats received either 2 mg/kg AMPH or saline and at 2,6, or 16 days post-injury, the 2-DG procedure was implemented. Autoradiograms were quantified by computer-assisted microdensitometry (CAM). Several authors have noted the difficulties in interpreting 2-DG data from injured brain; these problems are not present with CYOH. Therefore, we are statistically analyzing glucose utilization rates and comparing the same time points and experimental manipulations also using CAM of CYOH. (1) Sutton et al., Soc. Neurosci. Abstr. 12:1404, 1986. Supported by U.S. Army Contract No. DAMD17-86-C-6144.

399.6

MAINTENANCE OF CEREBRAL BLOOD FLOW DURING STIMULATION OF THE FASTIGIAL NUCLEUS IN RAT. D.M. Nitschke*, J.L. Williams, D.D. Heistad, and W.T. Talman. Lab of Neurobiology, VAMC and Univ. of Iowa, Iowa City, IA 52242.

Autoradiographic studies in the rat have suggested that electrical stimulation of the rostral fastigial nucleus (rFN) increases cerebral blood flow (CBF) without changing metabolism and impairs autoregulation. In contrast, using the pulsed Doppler and microsphere techniques in the cat, we have found only minimal changes in CBF during stimulation of the rFN. We have used microspheres to reassess the effects of rFN stimulation on CBF in the rat. In 7 chloralose-anesthetized rats, CBF was measured during a control period and during electrical stimulation of the rFN. Arterial blood gases were controlled and stimulus sites were confirmed histologically. Mean arterial pressure increased from 85 ± 5 to 109 ± 5 mmHg during stimulation ($p<.01$). Renal blood flow (in ml/100 gm/min) decreased from 691 ± 104 to 546 ± 100 ($p<.01$) while myocardial blood flow increased from 375 ± 36 to 579 ± 50 ($p<.01$). CBF did not change significantly (from 83 ± 13 to 99 ± 16). Cerebral vascular resistance increased by a mean of 9% (NS). This study suggests that stimulation of the rFN has a minimal effect on CBF during a moderate pressor response. The data 1) do not support the hypothesis that rFN has important effects on CBF and 2) suggest that autoregulation is maintained during rFN stimulation. Supported by HL32205, NS24621, and HL14388.

399.7

BIOLOGICAL AND METHODOLOGICAL IMPLICATIONS OF PROSTAGLANDIN INVOLVEMENT IN MOUSE BRAIN LIPID PEROXIDATION MEASUREMENTS. R. Bose*, G.R. Sutherland* and C. Pinsky* (SPON: G.B. Glavin). Depts. of Pharmacol. Therap. and Surgery*. U. of Manitoba, Winnipeg, MB, Canada R3E 0W3.

The cyclooxygenase inhibitor indomethacin [2.5 to 10 mg/kg intraperitoneally (ip)] lowered the measured values of thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde (MDA) equivalents, in mouse brain regions. At 10 mg/kg ip the inhibitor significantly reduced the estimated levels of MDA to 85% of those in saline-treated mice, in frontoparietal cortex and corpus striatum. In contrast, addition of indomethacin (50-800 ug/ml) in vitro to whole brain homogenates increased estimated TBARS in a concentration-related fashion. Such enhancement was not evident when indomethacin (200-800 ug/ml TRIS) was added to pure MDA standard solutions and must therefore reflect interaction of indomethacin with TBARS other than MDA. Hence, the contribution of prostaglandin metabolism-derived MDA, which could be as much as 15% in frontoparietal cortex and corpus striatum, should not be ignored when studying tissue lipid peroxidation.

Supported by Canadian Heart Foundation (GRS), Medical Research Council of Canada (CP), United Parkinson Fndn. (CP, RB), Alzheimer Society of Canada (CP), Cancer Res. Soc./Faculty of Medicine Program and Children's Hospital of Winnipeg Research Foundation Inc. (CP, GRS, RB).

399.8

PHARMACOLOGIC MANIPULATION OF NERVE BLOOD FLOW. D. W. Zochodne* and P. A. Low (SPON: P. J. Dyck). Neurophysiology Laboratory, Department of Neurology, Mayo Foundation, Rochester, MN 55905 USA

Peripheral nerve has both an epineurial (largely capacitative) and endoneurial (nutritive) blood supply but only the former is invested with sympathetic innervation. The importance of adrenergic control of vasa nervorum is unknown. Three techniques were used to study blood supply in the sciatic nerve of normal 300 gram Sprague-Dawley rats: NBF (nerve blood flow) by H_2 clearance with an endoneurial microelectrode; NBF by an epineurial laser doppler flowmetry probe; direct visualization and measurement of epineurial blood vessels with an integrated video-computer display system. Infusion of norepinephrine into the arterial supply of the sciatic nerve elevated systemic mean arterial pressure (MAP) but promptly shut down NBF in both the endoneurial and epineurial compartments. A preceding bolus of phentolamine blocked the fall in NBF without altering the systemic hypertensive response-NBF rose passively with the rise in MAP. Phentolamine alone significantly increased endoneurial NBF despite a fall in MAP. Epineurial norepinephrine bathing solutions had variable effects. Vasoconstriction was often confined to restricted vessel segments-other segments had arrest of the flowing RBC column. Little response was evident in presumed venules. These studies suggest that 'adrenergic tone' regulates NBF and that responsive vessel segments can adjust both nutritive and capacitative flow.

399.9

VARIATION IN THE VOLUME OF PERFUSED CAPILLARIES AND PERFUSED CAPILLARY SURFACE AREA AMONG CEREBRAL MICROVASCULAR BEDS. J. Fenstermacher, S.-Z. Lin*, A. Tajima*, and H. Nakata*. Dept. of Neurosurgery, SUNY, Stony Brook, NY 11794.

We have proposed and tested the hypothesis that the volume, surface area, and percentage of perfused capillaries varies among CNS structures. The experiments were performed on awake Sprague-Dawley rats using five radiotagged materials, quantitative autoradiography, and morphometric analysis of light micrographs. The following parameters were assessed: 1) local cerebral blood flow, 2) plasma, red cell, and blood volumes of perfused-labeled microvessels (PLM), 3) transfer rate constants and permeability-surface area (PS) products for several radiotracers, and 4) total capillary volume fraction (V_f) and surface area (S_f). Among the brain areas examined were the supraoptic nucleus (SON) and ventromedial nucleus of the hypothalamus (VMH). Based on the ratios of blood volume of PLM to capillary volume fraction, the volume percent of perfused-labeled microvessels, which are probably underestimated by this approach, ranged from 64% (SON) to 95% (VMH). Comparisons of PS products to total capillary surface area suggested that the percentage of capillary surface area perfused is least in the SON (about 35%) and greatest in the VMH (100%). Although the capillary volume and surface area percentages were somewhat different for the individual structures, the order from lowest to highest percentage was the same by both measures and supported the working hypothesis.

399.10

LACTATE-SUPPORTED SYNAPTIC FUNCTION IN THE RAT HIPPOCAMPAL SLICE PREPARATION. A. Schurr, C.A. West-Phelan* and B.M. Rigor, Department of Anesthesiology, University of Louisville School of Medicine, Louisville, KY 40292.

It is generally accepted that glucose is the primary substrate of both aerobic and anaerobic cerebral energy metabolism, while lactate is the end product of energy metabolism in the absence of oxygen. Recently, we have shown that both hyperglycemic levels of glucose and lactic acidosis not only are harmless but actually beneficial in experimental hypoxia in vitro (Brain Res. 421: 135, 1987; ibid. 438: 311, 1987). Hence, in the present study we tested the possibilty that cerebral energy metabolism can be fueled by lactate alone.

As a sole energy substrate, lactate supported evoked population spikes (synaptic function) in rat hippocampal slices for hours. Synaptically silent, glucose-depleted slices could be resuscitated with lactate to exhibit normal, glucose-like evoked population spikes. Glucose-supported synaptic function was abolished by 0.2 mM iodoacetic acid, while that supported by lactate was unaffected by the glycolytic inhibitor. Thus, under aerobic conditions lactate metabolism proceeds directly, via pyruvate, to enter the tricarboxylic acid cycle.

It is suggested that lactate accumulation as a result of certain conditions such as cerebral ischemia, could actually be beneficial to the ischemic tissue upon resuscitation, as this end product is capable of fueling energy metabolism.

399.11

GLUCOCORTICOIDS (GCs) MODULATE THE EFFECT OF PLASMA EPINEPHRINE ON REGIONAL CEREBRAL GLUCOSE UTILIZATION (rCMRgl). R.M. Bryan, Jr. and J.C. King*. Departments of Surgery (Neurosurgery) and Physiology, The M.S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033.

We tested the hypothesis that GCs modulate the effect of plasma epinephrine on rCMRgl. Rats were divided into four groups. In two groups, rats were adrenalectomized (ADX) three days prior to the experiment; in the remaining two groups, the adrenals remained intact (I). In one ADX and one I group, epinephrine was infused iv at a rate of 8 ug/kg/min; in the remaining ADX and I groups, an equal volume of saline was infused. Regional CMRgl was measured approximately 5 min after beginning the infusions. Mean arterial blood pressure increased from 102 mm Hg in the saline infused groups to 150 mm Hg in the epinephrine infused groups. Plasma glucose decreased as a result of ADX but was increased in both ADX and I groups that received epinephrine. Elevated plasma epinephrine had no effect on rCMRgl in the rats with intact adrenals. However, in the ADX rats, iv infusion of epinephrine increased rCMRgl in every brain region measured (13 of 17 regions statistically significant). Since the response to epinephrine was different depending on the presence or absence of GCs, we conclude that GCs can modulate the effects of plasma epinephrine on CMRgl.

399.12

GLYCOGEN ACCUMULATION IN CULTURED ASTROCYTES INDUCED BY GLUTAMATE AND METHIONINE SULFOXIMINE. R.A. SWANSON, A.C.H. YU*, F.R. SHARP, P.H. CHAN. Dept. of Neurology, VAMC and Univ. of California, San Francisco, CA 94121.

Methione sulfoximine (MSO) is known to increase brain glycogen *in vivo*, but is reported to have no effect in brain slice preparation (Folbergova J, J Neurochem 20:547, 1973). It has been proposed that the increased glycogen accumulation results from the induction of brain gluconeogenesis by MSO (Hevor TK *et al.*, J Cereb Blood Flow Metab 6:292, 1986). Using mature cultured astrocytes from rat cortex, 1 mM MSO added to the culture medium at the time of cell feeding increased glycogen content 150% measured 5 hrs later. MSO added at times after feeding had lesser effects on glycogen accumulation.

Since MSO inhibits several enzymes of glutamate metabolism, 1 mM glutamate was added to the culture medium. This also increased glycogen accumulation, with a maximal effect (100% increase) when given at the time of cell feeding. Both MSO and glutamate induced less than 50% increases in the astrocyte glucose content measured between 0 and 5 hrs after feeding.

These findings suggest that: 1) the mechanism of MSO-induced glycogen accumulation is related to its inhibition of glutamate metabolism; 2) gluconeogenesis is of insufficient magnitude to account for the glycogen accumulation (Cummins CJ *et al.*, J Neurochem 40:128, 1983); 3) it is possible that glutamate or a metabolite of glutamate may have a direct effect on glial glycogen metabolism.

399.13

GLUCOSE UTILIZATION AND LEUCINE INCORPORATION IN THE DEVELOPING RAT BRAIN. T.T. Soncrant*, H.W. Holloway*, D.M. Larson*, J.C. Miller* and S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

To study regional brain maturation, we measured local cerebral glucose utilization (LCGU) and regional leucine incorporation into brain protein (RLI) in developing male Fischer-344 rats. LCGU and RLI were measured in 80 brain regions, using quantitative autoradiography and [14C]deoxyglucose or [1-14C]leucine, in groups of 4-8 rats aged 7, 10, 15, 20, 25, 30, 35, 45, 60 and 90 days.

Whole brain glucose utilization rose progressively from 9.3±1.5 (S.E.) to 73.4±3.2 umol/100g/min between 7 and 45 days, then declined by 23% at 90 days (assuming no age change in the lumped constant). Early after birth (7-15 days), the LCGU:whole brain glucose utilization ratio was lower than the adult value in several forebrain regions (neocortex, medial cortex, caudate) and the inferior colliculus, and was higher in most other areas (hippocampus, amygdala, entorhinal cortex, globus pallidus, thalamus, hypothalamus, cerebellum, most brainstem areas). By 30 days of age, however, the pattern of LCGU approximated that of adult rats.

Whole brain leucine incorporation also increased progressively between 7 and 45 days, from 2.66±0.38 to 5.95±0.37 nmol/g tissue/min, then fell by 21% at 90 days. In many regions, the RLI:whole brain leucine incorporation ratio reached the adult value by 15 days of age. Although whole brain leucine incorporation paralleled glucose utilization during development, there was no correspondence between maturational time courses or adult values of LCGU and RLI.

The results demonstrate large and heterogeneous increases during maturation in indices of regional brain function and structure and indicate that rapid brain development continues until at least 45 days after birth in rats. Lack of correspondence between individual LCGU and RLI values suggests that they measure fundamentally different aspects of brain development.

399.14

ANOXIC TOLERANCE IN TURTLE BRAIN: LOWERED VULNERABILITY OR PREVENTION OF ENERGY FAILURE? C.P. Chih*, Z.C. Feng*, P.L. Lutz*, M. Rosenthal and T.J. Sick (SPON: R.E. Kelley). Div of BLR *RSMAS* and Dept of Neurology *Med Sch* Univ of Miami, Miami, Fl 33101

During prolonged anoxia, synaptic transmission is suppressed but ion transport activity is maintained in turtle brain. To determine whether ion homeostasis is maintained despite energy faiulre or by prevention of energy failure, high energy phosphates were measured in turtle brains subjected to anoxia and glycolytic inhibition (iodoacetic acid (IAA)). After 6 hours of anoxia, levels of ATP and extracellular K^+ were unchanged, although CrP was decreased by approximately 85% and lactate increased 9 fold. During simultaneous IAA superfusion and anoxia, ion homeostasis was lost in 1-2 hrs. Anoxic depolarization occurred when ATP was about 39% and CrP was about 25% of control. These results demonstrate that ion homeostais in turtle brain is maintained during anoxia only when ATP remains near control levels. The prolonged tolerance to brain anoxia in these animals appears due to mechanisms for avoiding energy failure rather than to lowered vulnerability to the consequences of energy failure. (Supported by NSF grant DCB8608670 and PHS grants NS14325 and HL38657)

399.15

UREMIA PRODUCES DIALYSIS-REVERSIBLE DEPRESSION OF GENICULATE BUT NOT SUPERIOR COLLICULAR OR CORTICAL 2-DEOXY-D-GLUCOSE UPTAKE. LIPMAN JJ, LAWRENCE P*, SULSER D*, WHITE DL*, TOLCHARD S*, AND TESCHAN PE*, VANDERBILT UNIV., DEPT. OF NEPHROLOGY, NASHVILLE, TN., USA.

Acute renal failure in humans and rats engenders a uremic syndrome of progressively deranged serum chemistry and neurobehavioral deficits. There is reduced EEG power with increased visual evoked potential latency. It is believed that the syndrome is engendered by the neurotoxic action of a dialytically removable uremic solute. To survey the central metabolic correlates of the phenomenon, rats were surgically implanted with chronic EEG electrodes, a peritoneal dialysis (PD) catheter guide and a jugular catheter. Nephrectomised bilaterally (NX), these animals were assigned to the following treatments: (A) attrition, n=10, whose uremia was unrelieved; (TD) therapeutic dialysis, n=11, 8 exchanges per day; (PUD), n=7, dialyzed as above with a solution containing elevated uremic levels of phosphate, and; (C) control n=10, sham operated, undialyzed. We find that in animals sacrificed 48 hours after NX regional cerebral glucose uptake (rCGLU) of attrition animals is depressed in the geniculate nuclei but not in cortex or superior colliculi. This is correlated with reduced EEG power and is reversed, in TD animals, by dialysis. Addition of phosphate to the dialysate blocked the restorative effect of dialysis on rCGLU and the EEG implicating this solute in uremia's neurotoxic effect.

INVERTEBRATE MOTOR FUNCTION

400.1

DEVELOPMENTAL CHANGES IN POSTSYNAPTIC NEURONS CAUSE LOSS OF A MONOSYNAPTIC REFLEX DURING METAMORPHOSIS IN MANDUCA SEXTA. J.C. Weeks and G.A. Jacobs. Dept. of Entomological Sciences, Univ. of California, Berkeley, CA 94720.

Manduca larvae exhibit a proleg withdrawal reflex mediated by direct synapses between proleg hair afferents and the proleg retractor motorneuron PPR. During the 4 d larval-pupal transformation ecdysteroid hormones cause PPR's dendritic arbor to regress substantially. Over the same time course the afferent-evoked excitation of PPR is severely attenuated; the compound EPSP evoked in PPR by a shock delivered to the sensory nerve is 70% smaller in pupae than in larvae. PPR's input resistance does not change significantly during this time. This decreased synaptic efficacy could be due to pre- and/or postsynaptic factors. The afferents change minimally during the larval-pupal transformation, suggesting that postsynaptic changes (e.g., PPR's regression) might be sufficient to cause loss of the reflex. This hypothesis was tested by using hormone treatments to generate heterochronic mosaic pupae which retained a larval proleg. In mosaic hemisegments the afferents remained in the larval state, while PPR regressed normally. The strength of the sensory-to-motorneuron pathway was attenuated to the same degree in mosaic hemisegments as in normal pupae. Thus, the loss of the withdrawal reflex appears due to developmental changes in postsynaptic rather than presynaptic neurons. Supported by NIH & Sloan grants to JCW, and an NIH-NRSA fellowship to GAJ.

400.2

DEVELOPMENTAL CHANGES IN PRE-ECDYSIS MOTOR PATTERNS OF THE MOTH, MANDUCA SEXTA. C.I. Miles and J.C. Weeks. Dept. of Entomol. Sci., Univ. of California, Berkeley, CA 94720.

Manduca exhibits a pre-ecdysis behavior just before ecdysis, when the old cuticle is shed at the end of a molt. Both pre-ecdysis and ecdysis behaviors are triggered by the peptide eclosion hormone (EH;1,2). Pre-ecdysis behavior consists of rhythmic compressions of the abdomen which are robust at molts between larval instars, but are no longer apparent at the larval-pupal molt. The only rhythmic movements at this time are weak dorsoventral flexions of the anteriormost abdominal segments. We have identified this behavior as a weakened version of larval pre-ecdysis by its timing, its dependence on EH, and the firing patterns of the motorneurons (MNs) which produce it. The strength of the motor pattern and the synaptic drive to pre-ecdysis MNs are greatly reduced at the larval-pupal molt. This is unlikely to be due to dendritic regression of the MNs, as they do not show significant structural changes at this time. Furthermore, the activity of these MNs during ecdysis, which immediately follows pre-ecdysis, is as robust as at larval molts. Thus, changes in the structure and/or functions of interneurons presynaptic to the pre-ecdysis MNs appear responsible for the weakening of this motor pattern at the larval-pupal molt. 1.Copenhaver & Truman '82, J. Insect Physiol. 28:695; 2.Truman et al. '81, Nature 291:70. Supported by an NIH fellowship to CIM and NIH and Sloan grants to JCW.

400.3

DESCENDING NEURONS RECEIVING COMMON SENSORY INPUTS DIVERGE FROM THE INSECT BRAIN TO FUNCTIONALLY DISTINCT MOTOR NEURON POOLS IN THORACIC GANGLIA. Nicholas J. Strausfeld and Jürgen J Milde. ARL Div. Neurobiol., Univ. of Arizona, Tucson, AZ 85721 and Dept. Zoology, Cologne University, Weyertal 112, D-5000 Cologne, FRG.

In *Calliphora*, intracellular fills reveal a variety of uniquely identifiable descending neurons (DNs), each distinguished by its characteristic dendritic morphology in the brain and by the morphology of its axon collaterals and terminals in segmental ganglia. Dendrites of specific DNs are structurally and functionally associated into clusters (DNCs: DN clusters), each forming the core of a discrete neuropil. A given DNC receives a characteristic set of primary mechanosensory afferents, terminals of visual interneurons, and interneurons derived from olfactory centers of the protocerebrum. In the brain, local and heterolateral interneurons appear to connect different DNCs in a fashion similar to interneurons linking groups of motor neuron dendrites in the thoracic ganglia. Although the same context-specific visual and mechanosensory stimuli can elicit responses from more than one member of a DNC, the axons of these neurons project to different targets. For example, axons from one known DNC diverge to functionally distinct groups of motor neurons belonging to the leg or the flight motor. Usually, however, the axon of any one DN gives rise to segmental collaterals which appear to invade motor neuron pools supplying segmentally homologous muscles. Examples are: branches to pro-, meso-, and metathoracic leg muscle motor neurons or branches to pro- and mesothoracic motor neurons supplying anterior dorsal neck muscles and direct flight muscles, respectively. **Supported by NIH Grant No. R01 EY07151-01**

400.4

MOTOR INNERVATION OF THE LOBSTER'S WALKING LEG MUSCLES. Theodore J. Wiens, Department of Zoology, University of Manitoba, Winnipeg, MB, Canada R3T 2N2.

The recent finding (Wiens, J. Neurobiol. 16, 183, 1985: Wiens and Rathmayer, J. Comp. Physiol. 156, 305, 1985) that the common inhibitor (CI) innervates all muscles in the walking legs of crabs and crayfish prompted a test of the same hypothesis for the much-studied Homarus americanus. Paired intracellular recordings were made from different combinations of muscles during stimulation of excitatory and inhibitory axons in various nerve branches of the leg. It was found that stimulation of CI in the efferent nerve entering the closer muscle can elicit IJP's in all nine muscles distal to the basipodite, as the hypothesis predicts (comp. Wiersma and Ripley, 1952) - but only if the leg nerve is intact distal to the coxa. Investigation of activation thresholds, IJP latencies, and effects of section suggested that CI splits into two branches in or proximal to the basipodite; one of these supplies the extensor, accessory flexor, bender, closer, and opener muscles in that order, and the other the reductor, flexor, stretcher, and rotator muscles. CI's two branches are thus disjoined in the autotomized leg. The rotator's only other efferent innervation comes from one of the flexor muscle's excitatory axons (comp. Sherman and Atwood, 1971). The opener receives a second inhibitory input from the well-described specific opener inhibitor (OI). These results resolve some questions about leg control and raise others.

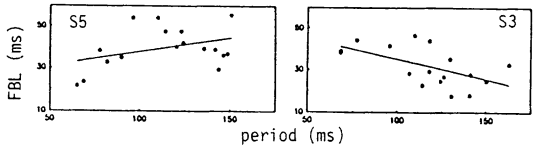
I thank C.K. Govind for discussions and equipment loans.

400.5

CRAYFISH SWIMMING: DIFFERENT EMG PATTERNS IN ROSTRAL AND CAUDAL ABDOMINAL SEGMENTS. D.H.Paul and R.K.Pye* Biology Depart., University of Victoria, BC V8W 2Y2, Canada

Swimming (nongiant tailflipping) in crayfish relies on a central pattern generator (CPG) whose nature is poorly understood (Reichert et al.,1981). Previous analyses of emgs from swimming crayfish and squat lobster found no corelationbetween flexor burst latency (FBL) and extensor burst period (EBP) in rostral segments (S2-4), but S5 FBLs in squat lobster covary with EBPs (Wilson & Paul,1987). We have now found that also in crayfish S5 FBLs and EBPs covary while concurrent S3 FBLs vary inversely with EBPs (Fig). Preliminary results suggest that intersegmental coordination varies with frequency and that this arises from differences in timing of extensor bursts, flexor bursts being nearly synchronous in S3 and S5 at all frequencies.

Thus nongiant tailflipping motor patterns are similar in crayfish and squat lobster. The underlying CPG appears to be fundamentally different than CPGs for locomotion using limbs in which similar segmental motor patterns are produced by coupled segmental CPGs.



400.6

THE NEURAL BASIS OF LIGHT-EVOKED WALKING IN CRAYFISH. T.W. Simon and D.H. Edwards, Dept. of Biology, Georgia State University, Atlanta, GA 30303

The backward walking response of crayfish to illumination of the Caudal Photoreceptor neurons (CPRs) in the 6th abdominal ganglion persists in restrained upside down animals. Leg movements in this position were sufficient to turn a walking wheel, and the responses appeared qualitatively similar to those in the same free-moving animals when ventrally illuminated. A cyclic response of Tonic Flexor (TF) motoneurons associated with backward walking (Kovac, 1974a,b; Moore & Larimer, 1987) commenced simultaneously with leg movements.

High frequency activity in a single CPR, similar to its light response, excited cells in the abdominal nerve cord which are part of a pattern initiating (PI) network for the TF response. When stimulated, some of these neurons could evoke a portion of the cyclic motor pattern; however, none were sufficient to elicit the entire TF response.

Interneuron A64 (Wine & Krasne, 1982), an identified mechanosensory interneuron, is also excited during the backward walking response. A64 is excited by unknown cells in the rostral portion of the abdominal nerve cord. It can excite TF motoneurons and may contribute to their excitation during the walking response.

In addition, swimmeret motoneurons and some hitherto unidentified cells which may contribute to extensor activation were excited as part of the walking response.

Both walking and the TF response to 6th ganglionic illumination persist following ligation of both circumesophageal connectives, and we are attempting to localize where the CPRs provide input to neurons which initiate walking.

400.7

EXCITATION AND INHIBITION OF THE CRAYFISH SWIMMERET RHYTHM BY STIMULATION OF SECOND NERVES OF THORACIC GANGLIA. A.Chrachri* (SPON: H.Anderson) Dept. of Zoology, Univ. California at Davis, Davis CA 95616.

In the crayfish, *Pacifastacus leniusculus,* the swimmeret motor pattern is strongly modified by tonic stimulation of the second nerves of each thoracic ganglion. Stimulation of these nerves at low intensity provokes a long-lasting activation of the swimmeret motor output. This stimulation increases the frequency of spikes of certain motor neurones within bursts in the swimmeret motor pattern, and also increases the amplitude of their membrane potential oscillation. At high intensity of stimulation of these same nerves inhibits the swimmeret rhythm. This inhibition is partially blocked by phentolamine, a competitive blocker of octopaminergic inhibition in the swimmeret system.

Blocking chemical synapses in these thoracic ganglia with low Ca^{++}- high Mg^{++} saline does not block these effects of second nerves stimulation, and Co^{++} backfills of thoracic second nerves reveal neurons in abdominal ganglion 1. Therefore, I suggest that these effects involve cell(s) which are located in ganglion 1, and that they act via pathways within the CNS, not via the blood. From the duration of their effects, these neurons might act through a modulatory process.

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400.8

CRAYFISH "BACKWARD WALKING" NEURONS INHIBIT LG COMMAND NEURON

D.H. Edwards, T.W. Simon, E.M. Leise & R.A. Fricke, Dept. of Biology, Georgia State University, Atlanta GA 30303

Tonic stimulation of individual neurons in the ventral lateral margin (VLM) of the crayfish abdominal nerve cord evokes a long-lasting discharge of abdominal tonic flexor (TF) motoneurons that is part of a backward walking response (Kovac, 1974; Moore & Larimer, 1987). We have found that stimulation of single VLM neurons, including A64, inhibits the LG command neurons for tailflip while they excite the TFs.

The VLM neurons inhibit LG by tonically depolarizing it. LG EPSPs evoked by sensory root stimulation are reduced in proportion to the level of LG tonic depolarization. LG EPSPs evoked by A64 are also reduced during the tonic depolarizing phase of the LG EPSP evoked by sensory root shock. Since many of the synapses onto LG are electrical, it is likely that LG inhibition results from the postsynaptic depolarization itself, perhaps by increasing LG membrane conductance or by back-biasing rectifying electrical synapses onto LG.

A64 produces a phasic burst followed by an 80 ms, high frequency (up to 300 Hz) spike train in response to phasic stimulation of the abdomen (Zucker, 1972). The phasic burst helps excite LG through monosynaptic electrical synapses. Our results suggest that the tonic phase of the response acts to inhibit LG by tonically depolarizing it. LG EPSPs from additional inputs would be reduced during this period, which coincides with recovery from the initial tailflip.

400.9

THORACIC OUTPUT OF CRAYFISH GIANT COMMAND NEURONES. W.J. Heitler and K. Fraser*. Gatty Marine Lab, Univ. St. Andrews, Scotland, KY16 8LB.

The crayfish escape tail-flip is initiated by 2 sets of giant command interneurones, MG and LG. The abdominal circuitry driven by these neurones has been extensively studied, but little is known about their thoracic output. We describe the anatomy and physiology of three identified segmental neurones in the 4th and 5th thoracic ganglia which receive direct input from the giant neurones.

1. Leg Promotor Motorneurone. This is driven 1:1 by the MG through a rectifying electrical synapse. It has powerful excitatory neuromuscular output, which shows initial massive antifacilitation, followed by slow facilitation. This is very similar to the output of the abdominal motor giant neurone.
2. Segmental Giant Neurone. This is driven 1:1 by the LG (TG5) and LG and MG (TG4) through rectifying electrical synapses. Its axon terminates close to the ganglion in numerous fine branches, which are located entirely within the nerve root. The SG drives fast flexor motorneurones of the trunk musculature.
3. Motor Giant Neurone. This is driven 1:1 by the LG through a rectifying electrical synapse.

All three neurones receive depolarizing IPSPs which can inhibit their input from the giant neurones.

The significance of these neurones will be discussed in relation to the possible evolution of the escape from a limb-driven to a trunk-driven behaviour.

400.10

ACTIVITY OF ABDOMINAL POSITIONING INTERNEURONS IS ALTERED BY TACTILE STIMULATION OF THE LOBSTER SWIMMERET. V.C. Kotak* and C.H. Page, Nelson Biol. Labs, Rutgers Univ., Piscataway, N.J. 08854.

We recently reported that mechanosensory stimulation of a swimmeret in lobster produces tonic abdominal extension (J.Comp.Physiol.158:225,161:695;J.Neurobiol.17:421). This includes excitation of the flexor inhibitor f5 and extensor excitor motoneurons and suppression of the extensor inhibitor e5 and the flexor excitor motoneurons. The present work characterizes interneurons that both drive (or inhibit) postural motoneurons and receive inputs from swimmeret mechanoreceptors. Flexion producing interneurons (FPIs) when current injected excited the flexor excitor motoneurons. Their activity was inhibited by mechanostimulation of feathered hairs, smooth hairs and integumentary receptors in the swimmeret, suggesting that FPI suppression by swimmeret inputs contributes in generating flexion inhibition/extension activity. However, since afferents are never inhibitory, the neural circuit for such responses must include at least one layer of interneurons between the afferents and FPIs. Extension producing (EPIs) when depolarized by current injection drive f5 and inhibit the flexor excitors. Since there was no 1:1 phase relationship between interneuronal and motoneuron responses to mechanostimulation of the swimmeret, a multisynaptic pathway between swimmeret afferents and tonic motoneurons must exist.

400.11

CHOLINERGIC / SEROTONERGIC SENSORY NEURONS HAVE BOTH CLASSICAL AND MODULATORY SYNAPTIC EFFECTS ON NEURONS IN THE CRAB STOMATOGASTRIC GANGLION. P.S. Katz, M.H. Eigg*, and R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

We have found a proprioceptive cell type in the stomatogastric nervous system of the crab, *Cancer borealis*, that not only provides fast synaptic input, but also has longer lasting modulatory effects on the bursting properties of central pattern generator neurons. We previously reported the existence of the GPR cells, a set of 4 muscle receptor cells in the gastro-pyloric region of the crab stomach (Katz and Harris-Warrick, Soc. Neurosci. Abstr. 13:821, 1987; 12:1207, 1986). These cells contain serotonin as determined by immunohistochemical staining and HPLC (Beltz et al., J.Exp.Biol., 109:35, 1984). We now show that they also have choline acetyltransferase activity, suggesting that serotonin and acetylcholine are cotransmitters in these cells. The GPR cells produce a variety of rapid synaptic effects in different stomatogastric neurons, including EPSPs of different time courses that are pharmocologically blocked by nicotinic antagonists. In addition, GPR activation causes prolonged modulatory effects in specific neurons, such as the induction of bursting and plateau potentials. These effects accumulate with repetition, and can outlast the duration of the stimulation by more than a minute. The GPR cells thus provide more than just a cycle-by-cycle correction of the motor output. Our results suggest that by altering the physiological state of some of the component neurons for extended periods of time, sensory input to central pattern generators can play a modulatory and instructive role.

(This work was supported by NIH NS 17323 and Hatch NYC-191410.)

400.12

INTERGANGLIONIC MOTOR ACTIVATION FROM THORACIC INTERNEURONS IN THE vGI SYSTEM OF THE COCKROACH. A.J. Pollack* and R.E. Ritzmann, Dept. of Biology, Case Western Reserve Univ., Cleveland, OH 44106.

The wind-mediated escape response of the cockroach Periplaneta americana has been studied as an example of a multi-element neural circuit capable of producing a complex behavior--a directed turn and run away from a wind source. Among the elements that have been studied are several metathoracic (T_3) interneurons which receive directional wind information from ventral giant interneurons (vGIs) and activate leg motor neurons in their ganglion of origin, T_3 (Pollack and Ritzmann, 1986). Other T_3 interneurons receive vGI inputs but do not excite leg motor neurons.

A large proportion of these interneurons send projections anteriorly to the mesothoracic ganglion (T_2) and beyond. Since the escape behavior involves coordinated movements among six multi-segmented legs, it is possible that intergangliónic projections exert control over motor outputs in other thoracic ganglia. We are examining this possibilty by observing motor outputs of both T_3 and T_2 simultaneously while stimulating a T_3 interneuron intracellularly.

Initial results suggest that interganglionic T_3 interneurons that produce motor outputs in T_3 also excite motor neurons in T_2. In some cases homologous motor neurons were excited in both ganglia, while in others motor neurons that are normally active in opposite phases of walking were excited in the two ganglia. T_3 interneurons that do not excite T_3 motor neurons also fail to excite T_2 motor neurons. We are continuing to characterize the T_3 and T_2 motor outputs as a result of T_3 interneuron stimulation in an attempt to further define this system and consolidate previous data which implicate specific T_3 interneurons as directional elements in the wind-mediated escape.

Supported by NIH grant NS 17411 to R.E.R.

400.13

INTERSEGMENTAL INTERNEURONES CONTROL THE GAIN OF LOCAL REFLEXES IN THE LOCUST. G. Laurent* and M. Burrows, Dept. of Zoology, University of Cambridge, CB2 3EJ England.

Intersegmental interneurones in a mesothoracic population receive mechanosensory inputs from a middle leg (Laurent, G. 1987 J. Neurosci. 7:2977-2989). They project ipsilaterally to the metathoracic ganglion where they make output connections (87% excitatory, 13% inhibitory) with premotor nonspiking local interneurones and with motor neurones controlling the movements of the ipsilateral hind leg. Metathoracic nonspiking local interneurones thus have receptive fields on both the ipsilateral hind and middle legs, the latter resulting from convergent excitatory and/or inhibitory inputs from several mesothoracic intersegmental interneurones. These nonspiking interneurones can gate or modulate metathoracic local reflexes when their membrane potential is manipulated experimentally. The inputs they receive from the intersegmental interneurones can have similar effects, implying that the gain of a metathoracic local reflex can be changed in accordance with information from the middle leg.

Supported by NIH grant NS 16058 to M.B.

400.14

TEMPERATURE EFFECTS ON SPONTANEOUS NEURAL ACTIVITY IN THE DRAGONFLY.
M. Kliss* and L. S. Stodieck. Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309.

Insects have often been represented as ideal poikilotherms, yet they are able to survive and function over a wide range of temperatures. It has generally been assumed that changes in behavior from temperature effects are related to changes that occur in neural processes. Previous investigations have shown that changes in temperature affect the conduction velocities of nerve fibers, as well as the afferent patterns of activity that arise in higher centers. The way in which the output of the nervous system compensates for these temperature induced changes in input, however, is not well understood. The present work focuses on the effects that ganglion temperature has on the neural activity in the dragonfly. Spontaneous extracellular recordings in the mesothoracic ganglion were obtained from both individual cells and groups of cells over a range of approximately 25 C. Spike frequency and spike slope were characterized for individual large cells. Interspike invertal histograms were obtained as a measure of total neural activity. Results indicated alterations in large cell spike frequency and slope as a function of temperature change. Changes in small cell activity were not as pronounced. Notably, the overall pattern of total neural activity remained relatively constant. The possible significance that temperature induced changes in spike slope may have on regulating neural output is discussed.

400.15

A BIOMECHANICAL ANALYSIS OF THE HEAD WAVING RESPONSE IN *APLYSIA* F. M. Kuenzi* and T. J. Carew. Dept. of Biology, Yale Univ., New Haven, CT 06520.

The head waving response (HWR) in *Aplysia* is a complex behavior that can be modified by operant conditioning (Cook and Carew, 1984, 1987). An analysis of the HWR and its plasticity requires an understanding of how the response is mechanically produced.

The *Aplysia* body is a soft, constant-volume cylinder, consisting of a muscular body wall enclosing a fluid filled space (hemocoel). Thus biomechanically, movement can be produced either by: 1) simple contraction and relaxation of appropriate muscles, or 2) contraction coupled with modulation of hydrostatic pressure. We investigated the invlovement of hydrostatic mechanisms in freely moving animals by cannulating their hemocoel and monitoring pressure with a solid state transducer. The basal pressure of resting animals was 1-2 cm of water. Three types of responses were measured: 1) Cyclic elevations and depressions of the head (in a locomotor rhythm) were associated with cyclic increases and decreases in pressure, respectively. 2) A variety of withdrawal reflexes were also all associated with clear pressure changes. 3) However, the HWR was *not* associated with any significant pressure change, and thus was due to muscle activity alone.

We next carried out a morphological analysis of the neck body wall musculature. We found muscle fiber bundles oriented longitudinally, circularly, and obliquely in three layers distinguishable by their connective tissue content and their fiber number. Having identified these muscle groups, it will now be possible to establish the innervation patterns of identified pedal motor neurons to assess their contribution both to the HWR and its modification by learning.

400.16

MODULATION OF APLYSIA RENAL PORE ACTIVITY BY L10, THE LUQ CELLS AND AN IDENTIFIED PERIPHERAL MOTONEURON.
J. Koester. Center for Neurobiology & Behavior, NY State Psychiatric Institute, and Columbia University, New York, NY 10032

Renal excretion in *Aplysia* begins with the filtration of blood across the wall of the heart. This ultrafiltrate is swept from the pericardium into the renal sac through a cilia-lined renal pericardial pore. Within the renal sac the fluid is elaborated into urine by excretion and absorption across the epithelium of the renal sac. The urine leaves the renal sac via the muscular renal pore near the posterior base of the gill.

Neuron L10, which is located in the abdominal ganglion, increases heart rate by exciting heart excitor cell RB-HE, so it may increase the rate of renal filtration. L10 also sends its major axon to the kidney. We previously showed that L10 excites the radial (opener) muscle of the pore and inhibits the circular (closer) muscle. When L10 fires, the opener muscle fibers generate synchronous twitches that do not follow the L10 spikes 1:1. Each time a twitch is generated, four events can be discriminated in an extracellular recording from the pore, in the following sequence: (1) L10's axon spike, (2) the muscle junction potential due to L10's direct connection, (3) a small action potential, which is attributed to a peripheral motoneuron, and (4) a large muscle action potential. Events (1) and (2) occur every time that L10 fires. Events (3) and (4) occur only when a twitch is generated, and they always occur together. I have localized event (3) to a single peripheral motoneuron that is embedded in the left side of the renal pore. Dye injection shows that this bipolar peripheral neuron, called the RP cell, sends processes completely around the circumference of the pore, presumably to mediate excitation of the radial muscle of the pore. Intracellular recording shows that the RP cell receives large EPSPs from L10 that must summate before an action potential is generated.

The peptidergic LUQ cells, L2-L6, are also located in the abdominal ganglion. We have previously shown that a subset of these cells inhibit the twitches that occur during pore opening. I have found that they also block events (3) and (4). Current work addresses the issue of whether the the inhibition of the RP cell by the LUQs is pre- or post-synaptic in origin. The RP cell provides a good vantage point from which to study the interaction of the central and peripheral nervous systems of *Aplysia*. (Supported by NIH grant NS14385).

400.17

PERIPHERAL ORGANIZATION OF THE SWIMMING SYSTEM IN A PTEROPOD MOLLUSC. R.A. Satterlie. Department of Zoology, Arizona State University, Tempe, AZ 85287-1501.

Locomotory structures in the pteropod mollusc Clione limacina include a pair of wing-like parapodia. Dorsal and ventral bending of the wings is accomplished by activation of four sheets of oblique muscle bundles, two associated with dorsal wing movement, and two with ventral wing movement. Wing muscle cells are simple, spindle-shaped cells up to 1.3mm in length and 30μm in diameter.

Intracellular recordings from muscle cells during swimming activity show graded junctional potentials and overshooting spike-like responses. Inhibitory junctional potentials have not been observed. Neuromuscular contacts are monosynaptic, and evidence for a peripheral motor nerve net is lacking. No difference has been noted in the electrical activity of slow-twitch and fast-twitch muscle cells.

Serotonin-like immunoreactive varicosities and processes have been found throughout the swimming muscle bundles. Since serotonin can trigger fast swimming in Clione, its role as a peripheral neuromodulator is being investigated.

400.18

ORGANIC AND INORGANIC CA++ CHANNEL BLOCKERS SHOW DIFFERENTIAL BLOCK OF PRAZIQUANTEL INDUCED MUSCLE CONTRACTION IN SCHISTOSOMA MANSONI . K. L. Blair*, J. L.Bennett* and R. A. Pax. Zoology, MSU, E.Lansing MI 48824.

Praziquantel (PZ) caused a rapid, sustained dose dependent contraction with an EC_{50} of 120 nM and a maximal effective dose (MED) of 1 uM. The Ca^{++} channel blockers nicardipine, Mg^{++}, and Cd^{++} shifted the PZ dose response curve to the left. Carbachol shifted PZ's ED_{50} up to 420 nM but did not affect the MED.

Nicardipine (10 uM) split the response into two phases, a fast transient component and a slower developing but sustained contraction. Mg^{++} blocked the sustained component while Cd^{++} blocked the transient component. When Mg^{++} and Cd^{++} were applied together, the entire contraction could be blocked.

These results are consistent with the hypothesis that PZ is a Ca^{++} channel activator. Carbachol appears to allosterically modulate the PZ activation. The differential effects of the three blockers indicates that more than one population of channel may be involved in the PZ induced contraction.

MOTOR SYSTEMS I

401.1

POSTNATAL GROWTH OF ALPHA- AND GAMMA-MOTONEURONS INNERVATING THE MEDIAL GASTROCNEMIUS MUSCLE OF THE CAT. He F.*, R.D. Guthrie* and W.E. Cameron. Depts. of Neurobiology, Anatomy and Cell Science and Pediatrics, Univ. of Pittsburgh Sch.of Med., Pittsburgh, PA 15261.

Medial gastrocnemius (MG) motoneurons were retrogradely labeled with horseradish peroxidase (HRP) following soak of the muscle nerve in cats of various ages. Lumbosacral spinal cord was sectioned horizontally and reacted with tetramethyl benzidine. The cell bodies and proximal dendrites of heavily labeled motoneurons were reconstructed.

	Ave. diam.		Surf. area		Volume	
Age	alpha	gamma	alpha	gamma	alpha	gamma
3 day	27.4	20.1	2047	1112	8433	3410
1 month	35.5	23.4	3383	1465	17829	5074
2 month	39.1	24.6	4082	1616	24012	5946
Adult	50.8	29.7	6937	2338	52326	10342

All values are means, N=250 for alpha; N=150 for gamma

The most rapid period of growth in both alpha and gamma motoneurons is between birth and 1 month of age. The rate of growth of the alpha-motoneurons was consistently greater than that of the gamma-motoneurons.

Supported by NIH grant HD22703.

401.2

MOVEMENT PATTERNS AND CYCLICITY OF NEWBORN RATS. C.R. Almli, J. Miller*, I. Orup* and R. Galiano*. Lab. Develop. Neuropsychobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

Research on early neurobehavioral development of movement (motility) patterns has revealed the existence of "cyclic," spontaneous movement patterns in a variety of species. Cyclic, spontaneous movement patterns have been described prior to birth or hatching, and after birth, and range in frequency from 1-3 minutes per cycle. However, there are questions concerning the locus of neural (brain and/or spinal cord) regulation of the cyclicity, and questions regarding the degree to which the movements themselves are "random" or "organized (functional precursors)." The present study used newborn rats and a computerized movement analysis system to quantify 12 characteristics of spontaneous movement (e.g., mouth, head, limbs, trunk, posture change, ambulation). The first spontaneous movements of the newborn rat were found to share a common cycle frequency (range: 0.5-1.0 cycles/min) for all body segments measured, suggesting a "cyclicity" generator in the brain and spinal cord. However, preliminary spinal transection data indicate a brain cyclicity generator, based on the loss of movement cyclicity caudal to the transection in newborn rats. The overall results also suggest that the spontaneous movements of newborn rats may be organized, and not random as previously thought. (NIH Animal Care Guidelines followed.)

401.3

PYRAMIDAL TRACT AXONS ARE INCREASED BY PRENATAL EXPOSURE TO ETHANOL. S. Al-Rabiai* and M.W. Miller (SPON: A. Gona). Dept. of Anatomy, Sch. of Osteopathic Med. and R.W. Johnson Med. Sch., UMDNJ, Piscataway, NJ 08854.

Rats prenatally exposed to ethanol have more corticospinal projection neurons than controls (Miller, J.C.N. 257:372, 1987), hence, we examined the number of pyramidal tract axons in Et-treated rats. Pregnant rats were fed a liquid ethanol-containing diet (Et), an isocaloric liquid control diet (Ct), or a diet of chow and water (Ch). On postnatal day 30, the offspring of these rats were sacrificed and their pyramidal tracts were processed for electron microscopy. 34% of the pyramidal tract axons in each group was myelinated. The axons were smaller and the myelin was thinner in Et-treated rats than in controls. The density of myelinated and non-myelinated axons was similar among the dietary groups, however, the overall size of the pyramidal tract was 10% smaller in Et-treated rats. Thus, the estimated number of axons in the pyramidal tracts of Ch- ($3.77x10^5$) and Ct-treated rats ($3.80x10^5$) was significantly greater than that of Et-treated rats ($3.18x10^5$). Nevertheless, because the brains of Et-treated rats are microcephalic this 15% decrease in absolute numbers is, in fact, a relative increase; other data show that layer V of Et-treated rats has 29% fewer neurons than that of Ct-treated rats (Potempa & Miller, this meeting). Thus, the ethanol-induced increase in the number of corticospinal projection neurons is matched by an increase in the number of pyramidal tract axons. Funded by AA06916 and DE07734.

401.4

INTRACELLULAR STUDY OF CORTICOSPINAL PROJECTION NEURONS IN RATS PRENATALLY EXPOSED TO ETHANOL. M.W. Miller, L. Klem*, N.L. Chiaia, and R.W. Rhoades. Department of Anatomy, School of Osteopathic Medicine and Robert Wood Johnson Medical School, UMDNJ, Piscataway, NJ 08854.

The effects of prenatal exposure on the structure and function of corticospinal projection neurons in mature somatosensory cortex was examined. Recordings were made in the 3 mo. old offspring of rats fed a liquid ethanol-containing diet (Et), an isocaloric liquid control diet (Ct), or chow and water (Ch). Corticospinal neurons were identified as cortical neurons antidromically driven by a stimulating electrode placed in the pyramidal decussation. Individual corticospinal neurons were injected intracellularly with horseradish peroxidase (HRP). Rats were sacrificed and the tissue was processed by standard HRP histochemical techniques. All intracellularly labeled neurons were pyramidal neurons. In Ct- and Ch-treated rats, the cell bodies of these neurons were distributed in layer V. In Et-treated rats, however, corticospinal neurons were distributed in layers II/III and VI as well as layer V. Sholl analyses of the basal dendrites of the corticospinal neurons in layer V showed that dendritic trees were significantly ($p<0.01$) more complex and more extensive in Et-treated rats than in controls. The conduction latency was significantly ($p<0.05$) shorter in Et-treated rats than in controls. These results show that prenatal exposure to ethanol has lasting effects upon the structure and function of cortical neurons. Funded by AA06916 and DE07734.

401.5

TRANSPLANTS ALTER THE DEVELOPMENT OF SENSORIMOTOR FUNCTION AFTER NEONATAL SPINAL CORD DAMAGE E. Kunkel-Bagden and B.S. Bregman Dept. Anat., Univ. MD. Sch. of Med., Balt. MD 21201

The aim of the current study was to determine if transplants (TP) of fetal spinal cord tissue alter the development of sensorimotor function after spinal cord injury at birth. Rat pups (2dpn) were hemisected (HX) at T6 (N=10). In one-half of the animals a TP of fetal (E14) spinal cord was placed into the lesion site (HX+TP, N=5). Postural reflexes were tested daily in lesioned (HX, HX+TP) and in unlesioned control (CON) rats from birth until 4 weeks of age. Posture and locomotion were observed, recorded on videotape and analyzed. The ability of the animals to right, climb onto a platform, and place in response to contact (CP), proprioceptive, and vestibular (VP) stimuli were assessed qualitatively and quantitatively. The posture, particularly hindlimb (HL) weight support and rotation, and the pattern of HL movement during locomotion was impaired in the HX group. In contrast, in the HX+TP animals these characteristics were similar to the controls. HX delayed the onset and maturation of the the postural reflexes that were immature at birth (righting, VP, CP). The presence of the TP prevented this lesion induced delay in the development of each of these, for example, righting: CON=6dpn, HX+TP=7dpn, and HX=10dpn. Transplants shift the timecourse of development of sensorimotor function toward normal. We suggest that this behavioral alteration is due to to the anatomical plasticity elicited by the transplant. Supported by NIH grant NS19259.

401.6

REFLEX PHYSIOLOGY AND CORRELATED MOTOR PERFORMANCE IN RATS WITH MILD CONTUSION SPINAL CORD INJURY.
F.J. Thompson, P.J. Reier, G.W. Schrimsher*, L.B. Jakeman, D.L. Winialski*, & C. Lucas. Dept of Neuroscience, University of Florida, Gainesville, Fl. 32610.

Our studies were aimed at examining neurophysiological parameters associated with spinal cord injury-related motor impairment in a rat model which previously has been characterized by correlative indices of behavior and anatomy.

Contusive injuries were produced using a modification of the Allen weight drop method and motor performance was quantified by the combined behavioral scoring (CBS) method of Wrathall and coworkers. Spinal reflex excitability was tested utilizing individually recorded H-reflexes. The extent of the spinal lesions was quantified by histological analysis. At one week post-injury we have observed that animals exhibited mean CBS scores of 74.0 (s.d.=22.0; n=5). Reflex amplitudes were not significantly different from controls (H/M ratio: 39.8, s.d.=13.6; n=6 compared to 48.4, s.d.=15.6, n=8). However, the sensory input required to achieve maximal reflex amplitudes in the injured animals was significantly less than in the normal animals. In addition, recovery curves of muscle and skin afferent-elicited reflexes suggested that presynaptic inhibitory depression of reflexes in the injured animals was significantly reduced compared to intact controls.

These studies suggest that motor impairment occurring within one week after contusion-injury of the rat spinal cord is most closely correlated with interactions that depend upon interneuron-mediated changes in primary afferent and motoneuron excitability. (Supported by NINCDS #NO1-NS-7-2300).

401.7

ACTIVITY OF CORTICAL NEURONS WITH TRANSIENT COLLATERAL PROJECTIONS TO THE CEREBELLUM. D.L. Tolbert, Dept. of Anat. and Neurobiol. and Surgery (Neurosurgery), St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

The stabilization or elimination of connections in the developing brain is correlated with impulse activity in converging afferents. Pathways which are normally eliminated will persist if activity in competing afferents is silenced. The transient collateral projection from the cerebral cortex to the cerebellum in neonatal cats was studied using electrophysiological techniques. Seven to 21 day-old kittens were anesthetized with alpha-chloralose and stimulating electrodes were positioned in the cerebellum (CB), pyramidal tract (PT) and medial lemniscus. Single unit recordings were made from the primary somatosensory cortex. Units with projections in the PT or CB or with collaterals in both structures (PT-CB) were identified by their antidromic activation. PT and/or CB projection neurons were not spontaneously active but fired only in response to antidromic activation. The projection neurons were capable of following relatively high frequency antidromic activation. Some units superficial to layer V projection neurons fired spontaneously and many were activated by cutaneous stimulation. Numerous units were synaptically activated following stimulation of the medial lemniscus but could not follow stimulus frequencies >1-2 Hz. The absence of activity in PT-CB projection neurons may contribute to the elimination of the collateral projection to the cerebellum. Support: NIH Grant NS20227.

401.8

Effect of Inferior Olive Lesion on Strychnine Seizure and Cerebellar [3H]AMPA Binding, M.C. Anderson*, E. Chung and M.H. Van Woert, Graduate Program in Neurobiology, Mt. Sinai School of Medicine, New York, NY 10029

Bilateral inferior olive (IO) lesions produce a proconvulsant state specific for strychnine-induced seizure and myoclonus in rats. Glutamate antagonists, glutamate diethylester (GDEE) and MK-801, both reversed IO lesion-induced leftward shift in the strychnine seizure dose-response curve. GDEE also inhibited strychnine-induced myoclonus in IO lesioned rats, while MK-801 had no effect on strychnine myoclonus. GDEE and MK-801 had no effect on strychnine-induced seizures and myoclonus in control rats.IO lesions produced a 30% increase in quisqualate (QA) displacable [3H]AMPA binding sites in cerebellar membranes. There was no difference in [3H]AMPA binding displacable by glutamate, kainate or GDEE. THe increase in QA sensitive [3H]AMPA sites may mediate the IO lesion induced proconvulsant state specific for strychnine-induced seizures and myoclonus. THis conclusion is consistent with the observation that QA antagonist pretreatment restored the threshold for both strychnine-induced seizure in IO lesioned rats to the control level, as well as significantly reducing strychnine myoclonus.

401.9

THE MATURATION OF AFFERENT PROJECTIONS TO THE RAT CEREBELLAR NUCLEI. Claude Gravel (a), Nicole Leclerc (a), Len Eisenman (b), and Richard Hawkes (a): a) Department of biochemistry and Laboratory of Neurobiology, Laval University, Quebec, PQ, Canada b) Department of Anatomy, Thomas Jefferson University, Philadelphia PA.

The corticonuclear projection in mammals is organized in the form of parasagittal bands of Purkinje (P) cells with discrete terminal fields in the deep cerebellar nuclei (DCN) and lateral verstibular nuclei. This organization can be revealed by using the monoclonal antibody anti-zebrin I (mabQ113). The olivocerebellar projection (OCP) is superimposed on this with cell clusters in the inferior olive contacting both specific P cell bands and cells in the deep nuclear target fields in the DCN and lateral vestibular nuclei. The different terminal fields in the DCN are already differentiated by birth, prior to the arrival of afferents from the Purkinje cells.

Anti-zebrin I has been used to study the maturation of the corticonuclear projection. The input matures surprisingly late: at P10 there are very few identifiable P cell terminals in the DCN. Instead, the P cell axons swirl around the periphery of the target fields, apparently unable to enter. Identifiable P cell terminals are only common from P14 onwards. Interestingly, anterograde tracer studies of the olivocerebellar projection reveals a very similar organization, with the climbing fiber axons intermeshed with the CNP at the periphery of the DCN.

This suggests two possible models by which the OCP can contact both its correct P cell band, and its correct nuclear target field. Either, both the OCP and the projection from a Purkinje cell compartment converge upon a common target territory in the DCN, and then the OCP fasciculates on P cell axons and follows them to the cortex (DCN specified), or the OCP recognizes a subset of P cell axons and birfurcates to the DCN in one direction and the cortex in the other (P cell specified).

401.10

LOCOMOTOR ACTIVITY EVOKED BY BRAINSTEM STIMULATION OF EMBRYONIC CHICK *IN OVO*. J.I. Valenzuela*, D.W. Ethell* & J.D. Steeves. Depts. Zoology & Anatomy, UBC, Vancouver, B.C., V6T 2A9

After thoracic spinal cord transection, as late as embryonic day 11 (E11), anatomical studies have provided evidence for axonal repair of brainstem-spinal projections (Nelson & Steeves, 1987, Soc. Neurosci. Abstr. **13**:972). To assess whether this axonal repair is also indicative of synaptic connectivity and functional recovery, *in ovo* techniques for focal electrical stimulation of brainstem locomotor regions in the decerebrate embryonic chick have been developed. After saturation of the amnionic fluid with Halothane anesthesia, each E18-20 chick embryo was mounted in a stereotaxic head holder and subsequently decerebrated. Leg muscle electromyographic recordings were used to monitor any evoked locomotor activity. Monopolar electrical stimulation (40-80 uA) of the caudal reticular formation, in control E18-20 embryos, evoked alternating and synchronous leg movements, characteristic of stepping and hatching motor programs, respectively. Using identical stimulation procedures, similar motor patterns were evoked in E18-20 embryos, that had their thoracic spinal cords previously transected on E6-11. (supported by MRC, Canada)

401.11

HINDLIMB MUSCLE SYNERGIES IN SPINAL TRANSECTED CHICK EMBRYOS. N.S. Bradley and A. Bekoff, Dept. EPO Biology, University of Colorado, Boulder, CO 80309.

Previously, we examined hindlimb muscle activity in intact chick embryos at 9-10 days (Bradley, N.S. & A. Bekoff, *Neurosci Abstr.* 13:1504, 1987). We now compare corresponding activity in spinal transected embryos to assess the role of descending neural input in the development of spinal networks for spontaneous motility.

White Leghorn embryos received low thoracic transections at day 3, and on day 10, 4-channel EMG recordings were obtained from one ankle extensor/flexor pair and one hip extensor/flexor pair during spontaneous motility *in ovo*. To characterize EMG records, cycle periods and burst durations were measured for all muscles, and burst onset latencies were referenced to the ankle extensor cycle period.

Similar to findings in intact embryos, motility is characterized by cyclic bursts in one or more muscles, and there are no differences in cycle period or burst durations across muscles. When two or more muscles are active, synergist pairs (*e.g.*, ankle and hip extensors) are coactive and extensor/flexor pairs are reciprocally active. While burst onsets for synergist pairs are closely related, burst durations are not closely related to either their own cycle period or the ankle extensor cycle period, but are closely related to ankle extensor burst duration. Further, and in contrast to intact embryos, burst durations for synergist pairs are closely related to each other. Also, spinal embryos typically exhibit shorter cycle periods and more cycles/episode than intact embryos.

These findings suggest that while muscle synergies for embryonic motility develop in the absence of descending input, some temporal elements are altered. Supported by NIH grant 20310.

401.12

ISOPYCNIC CENTRIFUGATION EMPLOYING PERCOLL STEP GRADIENTS FOR THE SEPARATION OF MOUSE FETAL MOTOR NEURONS FROM SPINAL CORD HOMOGENATES. M.J.Strong*, R.M. Garruto* and D.C. Gajdusek. Lab. of Central Nerv. System Studies, Natl. Inst. Health, Bethesda, MD 20892

We have developed a technique employing the principles of isopycnic centrifugation for the rapid isolation of a choline acetyltransferase (CAT) enriched cell fraction from fetal mouse spinal cord homogenates for studies of neurofilament catabolism in motor neurons.

Pooled spinal cords of fetal NIH:Or mice aged day 12-14 are dissociated, the suspension layered onto discontinuous Percoll step gradients (densities 1.040, 1.050, 1.060) formed in polycarbonate tubes, and centrifuged at 800 rpm x 15 min at 4°C in a Sorval swing-out rotor. The distinct cell populations collected at each interface can be directly plated without a wash step, enhancing cell yields and viability (85-95%). Data will be presented to demonstrate the concentration of CAT activity in the first interface. These cells, grown on a combined gelatin-polylysine-laminin substrate in serum-free medium over muscle, rapidly adhere and extend processes, producing large (30-70 µm), predominantly unipolar neurons with the morphological features of motor neurons. The second, CAT-poor fraction, yields nerve growth factor-sensitive, small (10-25 µm), multipolar neurons which are neither laminin or muscle dependant for survival. The final interface yields a mixed population of predominantly nonneuronal cells as demonstrated by immunohistology.

401.13

A MORPHOLOGICAL COMPARISON OF CHOLINERGIC NEURONS IN MOUSE SPINAL EXPLANT CULTURES WITH THOSE OF SPINAL TISSUE TAKEN FROM INTACT ANIMALS OF THE SAME LITTER. M. S. Pidnerney, D.L. Gonzalez, M.H. Droge, Dept. of Biology, Texas Woman's Univ., Denton, TX 76204.

Our objective is to use long-term explant cultures of spinal tissue to establish a model system for investigating the cellular/network mechanisms of motor pattern generation in mammals. Experiments are being conducted on tissue explant cultures and on tissue taken from intact animals to determine how closely the explants represent the in situ circuitry. Recordings of the motor activity in tissue explants are being pursued as a separate project. The aim of the current abstract is to compare the cholinergic cell populations in the lumbar ventral horn of fetal Balb/C mice (13 - 14 day gestation) with explant cultures of such tissue taken from the same litter.

Transverse sections of lumbar tissue (200 - 500 µm) taken from 13 - 14 day gestation mice have been established as explant cultures using a roller tube technique as described by Gahwiler (J. Neurosci. Meth. 4: 329-342, 1981). After a minimum of three weeks in culture, tissue explants were removed for identification of ventral horn cholinergic neurons using Karnovsky's acetylcholinesterase (AchE) staining method as modified by Guthrie et al. (B. Res. 420: 313-323, 1987). Explants were incubated in the impermeant AchE inhibitor phospholine iodide, fixed with buffered paraformaldehyde, and incubated in acetylthiocholine iodide staining solution. A parallel series of experiments was carried out on intact lumbar tissue taken from a littermate on the same day of culturing. Total cholinergic cell counts, cell density measurements and somal size distributions are being compiled using a Zeiss Videoplan Image Analysis System to quantify the cell loss due to the explantation and culturing process.

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401.14

THE DEVELOPMENT OF FAST AVOIDANCE BEHAVIOR IN XENOPUS. P. van Mier and R. Stoop, Dept. of Medical Physics & Biophysics, Univ. of Nijmegen, Nijmegen, The Netherlands.

During development, in the early swimming stage (stages 28-33) Xenopus embryos start to swim when they are either stimulated mechanically, chemically or by dimming the light. In general the direction of swimming seems random with respect to the applied stimulus. During metamorphosis larvae clearly start to show avoidance behavior and swim readily away from a given stimulus. In this study we present evidence that fast avoidance behavior develops shortly after the early swimming stage. To establish when and how this type of behavior develops in Xenopus embryos and larvae (stages 35-66) we used behavioral tests, high speed filming and electrophysiological techniques. Between stages 40 and 46 embryos and larvae started to respond to sudden external (especially vibrational) stimuli with a fast and powerful tail flip causing the animals to move away from the stimulus. It occurred that at stage 46 93% of the larvae responded as such to vibrational stimuli with a maximum sensitivity for vibrations around 1000 Hz. At stage 60 the maximum sensitivity had shifted to vibrations of approximately 25 Hz. High speed filming revealed that fast avoidance behavior usually started with an initial 'C'-shaped bending of the entire body. Simultaneous in-vitro stimulation of the lateral line nerve or the otic vesicle and electrophysiological recordings in brain stem and spinal cord suggested that the Mauthner cell might be involved in fast avoidance behavior in Xenopus larvae.

401.15

IDENTIFIED INTERNEURONS IN LARVAL AND PUPAL ABDOMINAL GANGLIA OF THE TOBACCO HORNWORM, MANDUCA SEXTA. D.J. Sandstrom and J.C. Weeks. Graduate Group in Neurobiology and Dept. of Entomological Sciences, Univ. of California, Berkeley, CA 94720.

In Manduca, the physiology, anatomy and developmental fate of many motorneurons (MNs) are known, whereas virtually nothing is known about interneurons (INs) presynaptic to the MNs. An isolated, desheathed ganglion preparation has greatly facilitated recording from small INs and has allowed pairwise recordings from INs and MNs. A number of INs presynaptic to proleg MNs have been identified by recording from the proleg motor nerve in larvae, while stimulating INs intracellularly. Many of these INs (>10) are reidentifiable based on their physiological and anatomical properties. In pairwise recordings, some INs produce unitary PSPs of short, constant latency in proleg MNs. As the larva progresses to the pupal stage, the proleg muscles degenerate and the MNs which innervate them regress dramatically. A number of the larval INs can be reidentified in pupae which, unlike the MNs, do not change drastically in morphology. We are currently comparing the physiology of the IN-to-MN synapses in larvae and pupae to gain insight into developmental changes in motor circuits. Supported by an NSF fellowship to DJS and NIH and Sloan grants to JCW.

401.16

INTERNEURONS INVOLVED IN MULTISEGMENTAL REFLEXES IN LARVAE AND PUPAE OF THE MOTH *MANDUCA SEXTA*. B.Waldrop and R.B.Levine. ARL Div. Neurobiology, Univ. of Arizona, Tucson, AZ 85721

We are studying the postembryonic development of neural circuits involved in the gin trap reflex of *Manduca*. The gin trap is a pupa-specific structure containing mechanosensory hairs which cause the trap to be closed by evoking a powerful contraction of ipsilateral muscles. The sensory neurons, motor neurons and muscles of the gin trap are retained from the larva where they mediate a different reflex. Since the terminals of the sensory neurons and the dendrites of the motor neurons are in different ganglia, there must be interganglionic interneurons carrying the sensory information. We are attempting to identify these interneurons and to determine if they are also retained and modified during metamorphosis.

The experimental preparation consists of an isolated abdominal nerve cord from either a larva or a pupa. Electrical stimulation of the sensory nerve leads to stage-specific response patterns recorded intra- and extracellularly in motor neurons. Interneurons are impaled and tested for responses to the sensory stimulus, and for their ability to evoke PSPs or changes in the spike rates of the motor neurons. We have found interneurons in the larva and the pupa which satisfy both of these criteria. Intracellular cobalt fills have revealed both dorsal and ventral dendrites and interganglionic axons, consistent with a role for these interneurons in sensory-motor integration in the gin trap reflex.

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401.17

REORGANIZATION OF AN IDENTIFIED LEG MOTOR NEURON DURING METAMORPHOSIS OF THE MOTH MANDUCA SEXTA. K.S. Kent and R.B. Levine. ARL Div. Neurobiol., Univ. Ariz., Tucson, AZ 85721

Using persistent fluorescent dyes (Fluoro-Gold, DiI) we are able to retrogradely label larval thoracic leg motor neurons (MNs) so that we can locate and characterize them with intracellular methods at later stages of development. We have focused our studies on one MN, which innervates the larval femoral flexor muscle and survives metamorphosis to innervate the femoral extensor muscle of the adult. During larval stages this MN has two distinct regions of dendritic branching, one located laterally in the leg neuropil and the other more dorsal and medial. Dendrites in both regions begin to regress during prepupal stages at the same time that peripheral terminals and target muscle begin to degenerate. Peripheral terminals are reduced to a few blunt, blebby processes that appear to remain associated with remnants of larval muscle and apodemes. Dendritic regression continues for three days following pupation until the dorso-medial arborizations are lost entirely and the lateral dendrites are reduced to a few sparsely branching processes. Over the ensuing few days, new afferent inputs reach the CNS and new muscles begin to form in the adult leg. Peripheral axons sprout new terminal processes and new dendrites begin to grow in the lateral region. To determine if the growth of new dendrites is influenced by ingrowth of new sensory inputs or contact with the new target muscle, we labeled the MN with DiI and removed the larval leg several days later, so that development proceeded in the absence of a new adult leg. Under such deprived conditions, the MN survives, loses larval dendrites, and begins to grow new adult dendrites on schedule. (Supported by NIH #NS24822).

TRANSPLANTATION I

402.1

IMMUNOLOGICAL REACTIONS IN INTRAVENTRICULAR XENOGRAFTS AND ALLOGRAFTS. Maciej Poltorak and William J. Freed NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032

We have compared the immunological reactions to embryonic brain tissue allografts and xenografts in the lateral ventricle of the rat brain, on the basis of distribution of immunocytochemical staining with antibodies against Ia antigen and helper/inducer cells. Host animals were randomly-bred Sprague-Dawley rats. The donor cerebellar tissues were taken from C57/BL mouse embryos or from Sprague-Dawley rat embryos, and implanted into the lateral ventricle of the host brain. Indirect immunofluorescence was performed on fresh frozen sections after 2,4 and 6 weeks of survival. Massive infiltrations of both cell types were found within xenografts. Ia antigen immunoreactivity was also present in the walls of small vessels near the transplanted tissue. This reaction was associated with destruction of the xenografts. In contrast, the allografts survived but nevertheless numerous cells expressing Ia- and helper-T-cell-antigen were usually detectable. Both Ia and helper cells were found along the injection needle tract and surrounding the graft 2 weeks after implantation. Some staining was also observed in small areas within the grafts. Both Ia and helper-T immunoreactivity decreased with the time of survival. In xenografts and to a lesser extent in allografts Ia immunoreactivity was enhanced in a population of small, rod-like, oblong cells, with tiny processes. These cells were found mainly within the white matter and their appearance corresponds to that of the microglia. These results suggest that the process of implantation of tissue and the associated brain injury induces enhanced Ia immunoreactivity in brain parenchyma surrounding both allo- and xenografts. Despite this predisposition to immunological reactions, only in xenografts did this reaction proceed through all of the steps required for a graft rejection response.

402.2

STUDIES OF HUMAN FETAL NEURAL TISSUE IN CULTURE. J.N. Barrett, E.H. Kriek, D. Nonner*, J.C. Kawamoto*, C. Kruse*, S. Waldrup*, D. Bhaskaran, and C.R. Freed, Depts. of Med. and Pharm., Univ. of Colo. Sch. Med., Denver, CO 80262 and Dept. of Physiol., U. Miami Sch. Med., Miami, FL 33101.

Human fetal tissue of 8 to 19 weeks' gestation was obtained from therapeutic abortions with consent given by the mother. Tissues included dopamine cells from ventral mesencephalon, cholinergic cells from basal forebrain, dorsal root ganglia, and adrenal medullary cells. Results showed that fetal adrenal medullary tissue from all gestational ages survived in the presence of mouse NGF or in cocultures with rat hippocampus, rat cortex or mitomycin C arrested C-6 glioma cells. Processes from those clusters were extensive. Clusters and processes were tyrosine hydroxylase positive and HPLC assay showed high concentrations of norepinehprine with lower concentrations of epinephrine and dopamine. Dorsal root ganglia showed neurite outgrowth in response to mouse NGF. Dopamine cells from ventral mesencephalon could be cultured only from fetus of gestational age 12 weeks or less. Cells showed TH immunoreactivity and dopamine production could be measured by HPLC. 1/5 preparations of basal forebrain showed stimulation of choline acetyl transferase activity following mouse NGF treatment. These data indicate that human fetal neural tissue can grow in tissue culture and that cells from adrenal medulla and central cholinergic systems are responsive to NGF-like growth factors.

402.3

GNRH AXONS FROM THIRD VENTRICULAR GRAFTS DO NOT REQUIRE ARCUATE NEURONS FOR TARGET LOCATION. R. Silverman, A.J. Silverman, H.M. Charlton,* M.J. Gibson. Columbia University N.Y., N.Y. 10032

Hypogonadal mice carry a mutation in the gene for gonadotropin releasing hormone (GnRH), resulting in infertility. Preoptic area grafts containing GnRH neurons from normal fetuses placed in the third ventricle of hpg mice result in increased indices of fertility subsequent to fiber outgrowth from graft into host target tissue. Immunocytochemically identified GnRH fibers grow from graft through host arcuate nucleus (A) to median eminence, the target. Neurons of A may play a role in this outgrowth. Monosodium glutamate has been shown to destroy 60-90% of A neurons. Would GnRH fibers grow through the MSG lesioned A in their typical manner or would the pattern be disrupted? Twenty neonatal hpg mice were given i.p. injections of 4mg/kg MSG and received grafts as adults: controls received saline. Thirty days after grafting, brain sections were stained immunocytochemically for GnRH. Tyrosine hydroxylase defined the A. Despite substantial neuronal loss, GnRH axonal outgrowth in the lesioned brains was indistinguishable in both pattern and intensity from that in the control brains. Similarly, the degree of reproductive recovery did not vary between groups. The majority of A neurons are not necessary for neurite outgrowth or pathfinding in this transplant system.

USPHS NS 20335 and HD 10665

402.4

EFFECTS OF CHRONIC GM_1 ADMINISTRATION ON TRANSPLANT-INDUCED REVERSAL OF MEMORY IMPAIRMENTS IN NBM LESIONED RATS. V. Haroutunian, A.C. Santucci, R. Gluck and K.L. Davis. Bronx VAMC & Mt. Sinai School of Medicine, New York, N.Y. 10468.

Both fetal cell transplants and the monosialoganglioside GM_1 aid recovery of function from several types of brain damage. We have studied the possible synergistic effects of these two approaches towards amelioration of n. basalis of Meynert (nbM) lesion-induced memory deficits. Seven to ten days after ibotenic acid-induced lesions (5ug in 1ul) of the nbM different groups of rats received either sham operations or septal area fetal (17mm) cell suspension ($2x10^7$cells) transplants (TR) into the frontal cortex. Beginning immediately after the transplant session different sub-groups then received daily IP injections of either saline or 20mg/kg GM_1 for 10 days. After the termination of the GM_1 treatments each rat was tested for the 72 hour retention of one trial passive avoidance. Saline injected nbM lesioned and nbM+TR rats performed poorly relative to sham lesioned rats ($ps<0.05$). The administration of GM_1 to nbM and nbM+TR rats normalized retention test performance to a level equivalent to sham operated rats ($ps>0.1$). Experiments now underway aim to determine the longevity and histochemical characteristics of this effect.

402.5

HOST-GRAFT INTEGRATION: RAT PERIAQUEDUCTAL GRAY AND ISOLATED BOVINE CHROMAFFIN CELL IMPLANTS J.D. Ortega*, J. Sagen, and G.D. Pappas (SPON: C.H. Anderson). Dept. of Anatomy and Cell Biology, Univ. of IL at Chicago, Chicago, IL 60612.

Previous work in this lab has demonstrated survival of rat adrenal medullary tissue following transplantation into rat periaqueductal gray (PAG), an important pain modulating center. Animals with either whole-tissue or isolated chromaffin cell transplants showed decreased pain sensitivity measured by standard analgesiometric tests following subcutaneous injection of nicotine. EM studies of the solid tissue transplants revealed heavy collagen encapsulation and gliotic shielding response at the host-graft border. Though isolation of graft from host does not appear to prevent diffusion of neuromodulatory substances into surrounding tissue, physical integration may be limited to some extent by these barriers. Furthermore, adrenal medullary tissue contains many cell types in addition to chromaffin cells which may interfere with host-graft integration. To circumvent this, isolated bovine chromaffin cell suspensions were used. Cells were grafted into the PAG of adult rats and examined morphologically at various intervals following surgery. Immunocytochemical analysis of the transplant site revealed the existence of healthy chromaffin cells which stain positive for tyrosine hydroxylase. At the host-graft border, both collagen encapsulation and gliotic shielding were virtually absent. Numerous synaptic contacts between the transplanted chromaffin cells and host processes were seen as early as two weeks following transplantation. In contrast to solid tissue transplants, lymphocytic infiltration was not observed, indicating a minimal immune response to the transplanted cells. Preliminary studies indicate increased cell survival and host-graft integration with constant intraventricular NGF infusion. (Supported by NIH grants NS25054 and GM37326).

402.6

THE EFFECT OF ADRENAL MEDULLARY TRANSPLANTS ON CSF ENKEPHALIN LEVELS AND PAIN SENSITIVITY. J.E. Kemmler* and J. Sagen, Dept. of Anatomy and Cell Biology, Univ. of IL at Chicago, Chicago, IL 60612.

Recent work in our laboratory has shown that it is possible to reduce pain sensitivity by transplanting adrenal medullary tissue into the subarachnoid space of the rat spinal cord. Chromaffin cells of the adrenal medulla store and secrete large amounts of opioid peptides and are therefore a potential reservoir for local release of these substances. The analgesia produced by stimulation of the transplanted adrenal medullary tissue was shown to be reversed by the opiate antagonist naloxone, suggesting that opioid peptides released from the transplants are important in mediating this analgesia. The aim of the present study was to more directly measure enkephalin release from the transplanted tissue using spinal cord superfusion techniques. Either adrenal medullary tissue or equal volumes of control muscle tissue was transplanted into the subarachnoid space of rat spinal cord. Pain sensitivity was assessed in transplanted animals using the tail flick, paw pinch, and hot plate tests. CSF samples were collected from transplanted animals using a push-pull superfusion apparatus. These samples were assayed for met-enkephalin using a radioimmunoassay. Basal levels of met-enkephalin release from the spinal cords of animals with control transplants was 7.8 pg/ml. This was nearly doubled to 14.8 pg/ml in animals with adrenal medullary transplants. The basal levels of met-enkephalin release were correlated with changes in pain sensitivity following adrenal medullary transplants. Nicotine stimulation further increased met-enkephalin levels in the superfusates. Results of this study indicate that adrenal medullary tranplants may serve as a locally available source of opioid peptides for the reduction of pain. (Supported in part by NIH grants NS25054 and GM37326).

402.7

CHROMAFFIN CELL GRAFTS INTO CNS PAIN MODULATORY REGIONS REDUCE PAIN SENSITIVITY IN A CHRONIC PAIN MODEL. H. Wang*, J. Sagen, and G.D. Pappas. Dept. of Anatomy and Cell Biology, Univ. of IL at Chicago, Chicago, IL 60612

Recent work in our laboratory has shown that transplants of either rat adrenal medullary tissue or isolated bovine chromaffin cell suspensions into the midbrain periaqueductal gray (PAG) or the spinal cord subarachnoid space are capable of decreasing pain sensitivity as assessed using acute analgesiometric tests (tail flick, paw pinch, and hot plate). However, therapeutic strategies effective in reducing response to these acute noxious stimuli cannot always be extrapolated to the treatment of chronic pain in humans. Therefore, the need for animal models for chronic pain which more closely represent human pain syndromes has been recognized. Currently available models include the adjuvent-induced arthritis model which results in a reversible hind-paw inflammatory arthritis in rats. Animals with hind-paw inflammation were transplanted with either solid rat adrenal medullary tissue, isolated bovine chromaffin cell suspensions, or control tissue into either the PAG or spinal cord subarachnoid space. Pain sensitivity was assessed in these animals by monitoring behavioral changes, particularly vocalizations. Baseline levels of vocalization responses were lower in animals with adrenal medullary and isolated chromaffin cell transplants than in animals with control transplants. The vocalizations were further reduced by 50% following the injection of nicotine in transplanted, but not control animals. Results of this study further suggest that the transplantation of isolated chromaffin cells into CNS pain modulatory regions may be an alternative approach toward the management of pain. (Supported in part by NIH grants NS25054 and GM37326).

402.8

THE USE OF A RETROVIRAL VECTOR TO IDENTIFY FOETAL STRIATAL NEURONES TRANSPLANTED INTO THE ADULT STRIATUM

P.C.Emson, S. Shoham, C.Feler[1], J. Price* & C.J. Wilson[1]
MRC Group, Dept. of Neuroendocrinology, AFRC Institute of Animal Physiology & Genetics Research, Cambridge CB2 4AT UK & [1]Dept. Anatomy & Neurobiology, University of Tennessee, Memphis.

The BAG retrovirus contains the E.Coli ß-galactosidase gene downstream of the viral long terminal repeat (LTR) sequence. In this study the BAG virus has been used to infect rat foetal striatal neurones before the infected neurones were transplanted into the ibotenate lesioned striatum of the adult rat.

After transplantation grafts were left in the host rat for eight months before the animals were anaesthetised, perfused and the brains processed to visualise bacterial ß-galactosidase activity. All the animals which had received infected neurones contained a number of surviving neurones some of which expressed the bacterial enzyme. The ability of foetal neurones to stably incorporate the virus and express the bacterial protein means that this method may provide a convenient route for the transfer of genetic information into neurones and would have potential in the treatment of neurological illness with transplants (Supported by a NATO collaborative research grant).

Price et al. (1987) Proc. Natl. Acad. Sci. 84, 156-160

402.9

FUNCTIONAL RECOVERY OF HIGH-AFFINITY CHOLINE UPTAKE IN THE HIPPOCAMPUS FOLLOWING SEPTAL CELL SUSPENSION TRANSPLANTS IN RATS WITH FIMBRIA-FORNIX LESIONS.

Y.Kaseda*, J.R.Simon, and W.C.Low. Depts of Physiology and Biophysics, Psychiatry, and Biochemistry, Inst. Psychiat. Res., and Program in Medical Neurobiology. Indiana Univ. Sch. of Medicine, Indpls., IN 46223.

The transplantation of cholinergic cells from fetal septal-diagonal band has been shown to innervate the hippocampus of rats with fimbria-fornix lesions, and to improve impaired learning and memory. It has been reported that AChE positive fibers grow from the graft into the host hippocampus. Electrophysiological properties similar to the normal septo-hippocampal cholinergic system have been seen between the graft and the host hippocampus. ChAT activity in the host hippocampus also appears to be normal. The purpose of this study was to determine the functional integrity of the cholinergic terminals derived from the graft. Cell suspensions were prepared from the septal-diagonal band area of 15-17 day old rat embryos. The transplanted group (TP) consisted of male Sprague-Dawley rats that received bilateral injections of 2 ul of cell suspensions into the dorsal hippocampus followed by bilateral fimbria-fornix lesions. Control rats received bilateral injections of 2 ul of vehicle into the dorsal hippocampus followed by bilateral fimbria-fornix lesions (LES) or sham lesions (SHAM). Neurochemical assays were performed 9-10 weeks after surgery. High-affinity choline uptake (HACU) (pmol/mg protein) in synaptosomes from dorsal and ventral hippocampus respectively was 20.1±1.7 and 23.3±0.9 in SHAM, 5.6±0.9 and 8.8±0.9 in LES, and 17.8±3.1 and 17.3±1.8 in TP. Thus, HACU was restored to 89 % and 74 % of control in the dorsal and ventral hippocampus respectively after transplantation. In addition, it was determined that the HACU related to the transplant could be activated by an elevated K^+ concentration as in normal cholinergic terminals. Thus, following preincubation at a K^+ concentration of 48 mM, HACU was activated by 30-40 % in all three groups. We conclude that the cholinergic terminals derived from the septal cell suspension have a functional HACU system, and that the HACU related to the graft can be activated by K^+ induced depolarization as in normal cholinergic terminals. (This work was supported by NIH grant RO1-NS-24464)

402.10

FETAL CNS TRANSPLANTS FOLLOWING TRAUMATIC BRAIN INJURY IN ADULT RATS. H.D. Soares* and T.K. McIntosh. (SPON: T. Koch). Surgical Research Center, Dept. of Surgery, Univ. of Connecticut Health Center, Farmington, CT 06032.

Adult rats subjected to lateral fluid-percussion (FP) traumatic brain injury (2.4 atm) exhibit parietal motor cortex necrosis resulting in neurological motor deficits. A cavity with glia limitans forms in damaged cortex between 2 to 4 weeks post-injury. We examined the ability of fetal cortical transplants to proliferate and survive at different stages of cavity development. Male Sprague-Dawley rats (350-450g) received a stereotaxic injection of whole fetal cortical tissue (E14 to E 16) into the cavity site at either 3 days (n=4), 5 days (n=5), 1 week (n=5), or 2 weeks (n=3) post-trauma. Animals were sacrificed at three weeks post-transplant and brains were subjected to either Nissl or acetylcholinesterase (AchE) histochemistry. AchE staining showed interlinking fibers between host and transplants at all timepoints. Some transplants grew to 4x original size and exhibited centralized areas of necrosis. Proximity of transplant to the left lateral ventricle appeared to be a determining factor in eventual transplant size. Transplants had fewer connections and more AchE positive cells than surrounding host areas. These data suggest that fetal brain transplants can survive and form connections when transplanted into injured cortex following traumatic brain injury. (Supported by VA Merit Review Grant 74R).

402.11

IS PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L) A USEFUL MARKER FOR LABELING NEURAL GRAFTS? A.M. Bhatia*, J.N. Kott, and L.E. Westrum. (SPON: P. Swanson) Depts. Neurol. Surg. and Biol. Struct., Univ. of Wash., Seattle, WA 98195.

We are presently evaluating different methods for labeling donor cells prior to neural transplantation in the olfactory system. Incubation of cultured fetal human spinal cord cells in PHA-L has been reported to allow discrimination of donor cells grafted into pre-lesioned adult rat cerebral cortex (Kamo et al., Neurosci. Lett. 76:163, 1987). We are incubating solid fetal olfactory bulbs of rat fetuses of embryonic ages 15-18 days in 1% PHA-L prior to direct transplantation into intact or pre-lesioned neonatal rat olfactory system. In addition to sections reacted for PHA-L by the ABC method, cell and fiber stains are used on alternate frozen sections. The cell and fiber stained sections show "viable grafts" with appropriate neuronal types and with fibers traversing the host-graft interface. However, the PHA-L labeling by this method appears to be incomplete, possibly due to insufficient penetration of the fetal tissue blocks. Further, host neurons at various sites outside of the graft may show PHA-L reactivity perhaps from uptake of label from degenerating donor cells. Use of cell suspensions rather than solid tissue blocks is planned next for study. (Supported by NIH Grants NS09678 and DE04942. LEW is an affiliate of CDMRC.)

402.12

ULTRASTRUCTURAL LOCALIZATION OF GOLD PARTICLES WITHIN CENTRAL GRAFTS LABELED WITH GOLD-FILLED SENDAI VIRAL ENVELOPES. J.N. Kott, L.E. Westrum, and [1]G.W. Arendash. Depts. Neurol. Surg. and Biol. Struct., Univ. of Washington, Seattle, WA 98195 and [1]Dept. Biol., Univ. of South Florida, Tampa, FL 33620.

Ardizzoni, Michaels and Arendash (Science 239:635, 1988) have described a method using gold-filled Sendai virus envelopes to mark cell suspensions prior to transplantation. We are examining similarly labeled nucleus basalis suspension grafts by electron microscopy to determine the distribution and character of this label within transplanted cells to assess the possibility of its use in studies of synaptology in graft tissue. Preliminary examination of transplanted labeled tissue in the immediate vicinity of the graft site has revealed gold particles associated with various cytoplasmic membranes of different cell types including neurons, glia and their processes. Unexpectedly, heavy labeling was observed within the nuclei of both neurons and glia. The gold particles vary between 15 and 80 nm diameter and appear as single round profiles or aggregates of several such profiles. While gold label was found throughout the limited area we have examined, its concentration dissipates with distance from the center of the graft. Examination of sites remote from such grafts, including pre-lesioned target areas is planned. (Supported by NIH Grants NS09678 and DE04942. LEW is an affiliate of CDMRC.)

402.13

FINE STRUCTURAL CORRELATES OF NEURAL TRANSPLANTATION. W. Wu* and D.E. Scott, Dept. of Anatomy and Cell Biology, Eastern Virginia Medical School, Norfolk, VA 23501.

This investigation has focused on basic questions of vascularization, survival, and regeneration in neurites in the transplanted endocrine hypothalamus. Normal fetal hypothalamic grafts 15 days post-coitus were transplanted into the third cerebral ventricle of host rats with diabetes insipidus (DI). Blood vessels were extant in grafts within the first week following transplantation. Vasopressin (AVP) positive neurons were observed in the grafts. AVP neurons project fibers which develop three types of neuroanatomical relationships with the host brain: 1) terminate in host median eminence; 2) terminate around the blood vessels which grow from the host brain into the graft; and, 3) terminate directly into the ventricular lumen of the host brain. Fenestrated capillaries with distinct perivascular spaces were observed in the ventral regions of the graft in proximity to the host median eminence. Four weeks following transplantation, AVP-positive neurons within the graft appeared well differentiated and exhibited numerous axodendritic and axosomatic synapses. This study indicates that the morphological relationships that develop between transplanted AVP-positive neurons and host-brain recipients may allow for AVP-positive neurons to release neuropeptide hormones into both the vascular and CSF compartments. Supported by NSF grant BNS 8709687.

402.14

THE PRESENCE OF A BLOOD-BRAIN BARRIER IN NEURAL XENOGRAFTS IS RELATED TO THE IMMUNOLOGICAL STATUS OF THE HOST. M.J. Young*, K. Rao, and R.D. Lund (SPON: M. Bennett). Dept. Neurobiol., Anat., and Cell Sci., Univ. of Pittsburgh Sch. Med. Pgh., PA 15261.

We are investigating immunological correlates of blood-brain barrier (BBB) breakdown in retinal xenografts in rats, utilizing skin grafting to initiate a timed immune response to the transplanted neural tissue. Embryonic (E13-14) CD-1 mouse retinae were grafted into the brainstem parenchyma of neonatal (P1) Spraque-Dawley rats. 21 days after transplantation one group of animals recieved a 1 cm^2 CD-1 skin graft on the flank to provoke an immune response to the neural graft. Control animals recieved no skin graft. 2-8 days post skin-grafting (DPSG) animals were injected in the femoral vein with horseradish peroxidase (HRP). Brains were processed for Nissl, HRP-TMB, and anti-M2, -M6, -Ia, -lymphocyte, -macrophage, and -astrocyte monoclonal antibodies.

Experimental and control animals injected 2-4 DPSG showed no leakage of HRP. A small percentage of 5 DPSG animals showed isolated, patchy leakage, but little or no evidence of rejection. At 6 DPSG over 50% of the grafts showed evidence of HRP leakage and the infiltration of lymphocytes. At 7-8 DPSG massive leakage of HRP and widespread infiltration of lymphocytes, macrophages, and astrocytes was evident.

The results demonstrate that the status of the BBB is closely correlated to the immunological status of the host. They also support the presence of a BBB in neural transplants, but suggest that immunological factors should be considered in allo- and xenogenic grafts of neural tissue. (NEI EY05283 & Winters Fdn)

402.15

SELECTIVE DENTATE GRANULE CELL LESIONS BY FLUID INJECTION. B.P. Vietje*, J. Wells and P.C. Camp*
(SPON: S.L. Freedman). Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

In our previous studies involving cell suspension transplants into the dentate granule cell layer, (GCL), it was noted that the fluid volume of the injected cell suspension split open the host tissue along the hippocampal cleavage planes, particularly along the hilus/GCL interface, resulting in axotomy and subsequent death of the granule cells. The present study was undertaken to study the effects of granule cell lesions in the absence of a transplant. Reproducible GCL lesions were created by stereotaxic injections of Hank's Balanced Salt Solution into the GCL of adult rats. After survival times of 6 days to 2 months, brains were analyzed by Timm's stain, nissl stains, AChE histochemistry, and GFAP and S-100 immunoreactivity. GCL lesions resulted in a loss of granule cells, decreased mossy fiber staining thinning of the stratum moleculare, and a marked gliosis surrounding the lesion. GCL lesions contribute to the altered host environment in transplant studies and provide an important control for separating the effects due to GCL lesions from those of the tgransplanted tissue. Additionally, specific granule cell lesions can be used to study the function and connectivity of the dentate granule cells. Supported by NS23266.

402.16

AUTORADIOGRAPHIC OBSERVATIONS AFTER OLFACTORY BULB TRANSPLANTATION. T. Zigova, P.P.C. Graziadei and A.G. Monti Graziadei. Dept. of Biology, Florida State Univ., Tallahassee, FL 32306-3050 and Inst. of Neurobiology, Kosice, Czechoslovakia.

In a previous study, after removal of the olfactory bulb in neonatal rats, we have transplanted embryonic presumptive olfactory bulb to see if the embryonic tissue would have interacted with the sensory input and the brain of the host. Since the transplanted olfactory bulb quite often developed acquiring the characteristics of a normal OB, it was important to demonstrate that the entire bulb was from the donor. In this study 3H-thymidine was injected to pregnant rats at the time when the large neurons of the bulb were undergoing cellular division. The autoradiographically labeled presumptive olfactory bulb from E14-E18 embryos was transplanted to neonatal P1-P5 pups in place of the removed olfactory bulb. Observations were carried on at postoperative survival times from 20 to 45 days. The results of this study have shown that the transplanted bulbs were capable of establishing connections with both the sensory input and the brain of the host.
Supported by NIH grant NS 29699 and NSF grant BNS 86 17022.

402.17

EFFECT OF CONCENTRATED ANTIBIOTIC SOLUTION ON SURVIVAL OF RAT FETAL DOPAMINERGIC NEURONS. J. K. Morgan*, R. J. Plunkett*, M. A. Palmatier, and E.H. Oldfield* (SPON: N. H. Spector). SNB, NINCDS, NIH, Bethesda, MD. 20892

Mesencephalon from aborted fetuses is a source of dopaminergic neurons which may be useful for implantation in Parkinsonian patients. A contraindication to the use of this tissue is the high rate of contamination with vaginal flora. We devised a technique to sterilize this tissue in a concentrated antibiotic wash. As a correlative study, we examined the possible cytotoxic effects of this concentrated antibiotic solution on cells from fetal rat mesencephalon. The ventral mesencephalon dissected from embryonic day 13 or 14 rat embryos were washed either in the concentrated antibiotic solution or PBS with 6% glucose. Cell suspensions were then prepared by papain digestion and mechanical dissociation. Initial viability was assessed by trypan blue exclusion and found to be > 80% in both groups. Survival of dopaminergic neurons in cell culture was documented by tyrosine hydroxylase immunohistochemistry. We conclude that a brief exposure of fetal tissue to the concentrated antibiotic solution has no apparent cytotoxic effect.

402.18

PHENOTYPIC SELECTION OF NEUROBLASTOMA CELLS FOR USE IN TRANSPLANTATION IN ANIMAL MODELS OF PARKINSON'S DISEASE. D.G.Walker*, B.E.Boyes*, P.L.McGeer and E.G.McGeer. Kinsmen Lab. of Neurol. Res. Univ. Brit. Col., Vancouver, B.C., Canada. V6T 1W5.

The feasibility of using differentiated neuroblastoma cells in long term neural grafting procedures has been demonstrated (Gash et al., Science,233:1420,1986). To follow up our previous work of transplantation of human fetal neurons in an animal model of Parkinson Disease (Kamo et al. Brain Res.397:372,1986), by developing a renewable cell source, subclones of IMR-32 cells with dopaminergic and adrenergic characteristics were isolated using a procedure of Breakefield and Nirenberg (PNAS,71:2530,1974). Cultures of unselected IMR-32 cells were grown in F-12 media, deficient in tyrosine, to select for cells producing tyrosine hydroxylase, tryptophan hydroxylase or phenylalanine hydroxylase. In this media, the majority of cells rapidly die, and isolated foci grow. Approximately 1 out of 8,000 uncloned IMR-32 cells can grow in tyrosine deficient media. Following three passages in tyrosine deficient media, 40 tyrosine independent subclones were isolated and established. Catecholamine and indoleamine content and specific activity of tyrosine hydroxylase in each isolate was measured by HPLC with electrochemical detection. Treatment of selected isolates with retinoic acid (10 μM) and aphidicolin (1 μg/ml) induced differentiation and neurite formation.

Sponsored by grants from the MRC (Canada) and B.C.M.S.F.

402.19

LONG-TERM MAINTENANCE OF ENCAPSULATED PC12 CELLS IN VITRO AND AS BRAIN IMPLANTS. C.B. Jaeger, S.R. Winn*, P. Aebischer* and L.A. Greene. Dept. Physiol. Biophys. NYU Med. Ctr., New York, NY 10016, Artificial Organ Lab. Brown University, and Dept. Pathol. Columbia University.

Replacement therapy of dopamine in the brain by tumorigenic catecholaminergic cell lines (TCL) requires enclosure and containment of such cells. We studied the survival and the presence of tyrosine hydroxylase (TH) of encapsulated TCL. One TCL, the PC12 pheochromocytoma cell line, known to synthesize high levels of dopamine, was incapsulated and implanted in the forebrain of rats, or maintained in vitro. Cell-filled permselective polyvinylchloride acrylic co-polymer capsules (Aebischer et al., ASAIO 10:96,'87) were fixed at intervals up to six months, the morphology of the cells was studied, and their enzymatic properties were examined by immunocytochemistry. PC12 cells remained healthy within the capsules. Viable cells were found adjacent to the capsule wall within a radius of 100 to 150 μm. Mitotic figures in some of the enclosed PC12 cells were taken as evidence for continued cell growth within the capsules. Mitosis was observed up to six month following capsule placement into the brain. Approximately ten layers of PC12 cells located adjacent to the capsule wall were TH positive in correspondence with the region of viable cells. We conclude that the procedure of encapsulation allows long-term implantation of TCL into the brain.

THE AGING PROCESS I

403.1

CHARACTERIZATION OF THE EFFECTS OF NUCLEUS BASALIS LESIONS IN RATS 14 MONTHS POST-LESION. R. D. Terry*[2], R.J. Mandel[1,2], G. Buzsaki[2], F.H. Gage[2] & L.J. Thal[1,2]. (Spon. R. B. Livingston). [1]Dept. Neurol. VAMC, SD, 92161, [2]Dept. Neurosci., UCSD, La Jolla, 92093.

Recently, rats with nucleus basalis magnocellularis (NBM) lesions 14 mo. previously, were reported to display neuropathology similar to that seen in human Alzheimer's brains (Arendash, et al., Science 238:952, 1987). We report that, using a similar excitotoxic lesion paradigm, we did not find similar neuropathology, although the rats were impaired on biochemical, behavioral, and electrophysiological indices.

Six F-344 male rats received bilateral ibotenate lesions of the NBM. Three weeks post-surgery lesioned and unlesioned controls (n=6) were tested in a water maze task (10 trial blocks, 1 block/day, 4 trials/day). Fourteen mo. later, 4 rats from each group were retested in the same water maze (3 trial blocks). Immediately after behavioral testing, these 8 rats were implanted with cortical electrodes for freely moving EEG recording. The rats were then sacrificed for histological examination with acetycholinesterase (AChE) histochemisty, cresyl violet, and Bielchovsky silver staining. The 4 remaining rats were sacrificed for measurement of cortical choline acetyltransferase (ChAT) activity.

The NBM lesioned rats had 22.3% depletion of cortical ChAT 14 mo. after lesioning relative to controls. The lesioned rats were impaired relative to controls on initial acquisition of the maze task but did reach equivalent levels of performance during trial blocks 6-10. Fourteen mo. later, lesioned rats were impaired relative to controls on retention and reacquisition of the maze task. Although the biochemical and behavioral deficits displayed by the lesioned rats were relatively mild, EEG recording revealed high amplitude waves which were absent in control rats. While AChE positive neurons in the NBM were reduced in the lesioned rats' brains, silver staining revealed no pathology resembling cortical plaques.

403.2

INCREASED EXTRA-ADRENAL CHROMAFFIN CELLS IN AGED FISCHER-344 RATS: AN IMMUNOCYTOCHEMICAL EVALUATION. G. Yang*, M.F. Matocha and S.I. Rapoport. Lab. of Neurosci, NIA, NIH, Bethesda, MD 20892.

The number of extra-adrenal chromaffin cells in male Fischer-344 rats is strikingly increased with age (Partanen, M. et al, Neurobiology of Aging 5:105-110, 1984). In this study, the mechanism of the senescent increase was addressed using immunocytochemical methods. A monoclonal antibody against the 5-bromo-2'-deoxyuridine was injected intraperitoneally (50mg/kg b.w., once a week for 14 weeks) into rats aged 26 months, and paraganglia were tested for incorporation of the uridine . None of the extra-adrenal chromaffin cells was labeled by the antibody, indicating that the age-related increase is not due to cell proliferation. To examine if a glucocorticoid receptor (GR) mechanism is involved in the senescent increase, the temporal pattern of GR immunoreactivity in the extra-adrenal chromaffin cells was followed and was compared with that in adrenal chromaffin cells. No detectable changes in immunoreactivity were found in extra-adrenal chromaffin cells, whereas the immunoreactivity decreased with age in adrenal chromaffin cells. The persistence of GR in extra-adrenal chromaffin cells and the correlation of GR immunoreactivity with distinct aging fates of the chromaffin cells suggest that the GR is involved in the numerical increase of extra adrenal chromaffin cells in the aging rats.

403.3

AGE-RELATED CHANGES IN ADRENAL TYROSINE HYDROXYLASE EXPRESSION AND CATECHOLAMINE CONTENT: EFFECTS OF DIETARY RESTRICTION. M.A. Moore,* C. Hale,* W.J. Burke,* H.J. Armbrecht,* and R. Strong* (SPON: D.F. Russell). Ger. Res. Ed. and Clin. Ctr., St. Louis VA Med. Ctr. and Depts. Pharmacol., Biochem., Neurol., and Med., St. Louis Univ. Schl. of Med., St. Louis, MO 63125

Dietary restriction of total calories increases both the median and maximum life span of rodents and attenuates various age-related biochemical changes. We hypothesized that dietary restriction may affect the rate of aging by altering programmed changes in gene expression. Adrenal medullary catecholamine metabolism increases markedly during aging. Therefore, we measured enzymes of catecholamine metabolism, choline acetyltransferase activity and catecholamine content in adrenal glands of food-restricted and ad lib fed rats of different ages. Tyrosine hydroxylase (TH) activity increased 2-3 fold from 2 to 23 months of age. These changes in TH were paralleled by increases in dopamine (DA) content. There were no changes in the other parameters measured. Contrary to expectations, lifelong dietary restriction failed to attenuate the effect of aging on TH or DA. In fact, food restriction increased tissue content of catecholamines and TH. Preliminary results show age-related increases in adrenal TH mRNA. We are currently evaluating dietary restriction on TH mRNA.

403.4

EFFECT OF LIPID PEROXIDATION ON GLUCOSE TRANSPORT IN ASTROCYTES: POTENTIATION BY ETHANOL. T-C Jou*[1], S-M Liu*[1], FF Ahmad*[2], DL Cowan*[3] and AY Sun[2]. [1]Institute of Neuroscience, National Yang-Ming Medical College, Taipei Taiwan, ROC, and Departments of [2]Biochemistry and [3]Physics, University of Missouri, Columbia, MO 65203.

Ethanol has been shown to enhance lipid peroxidation in vivo and in vitro. We used cell culture as a model to study the metabolic regulation of the central nervous system along with the spin trapping technique using electron spin resonance spectroscopy to directly detect free radicals in the system. In presence of low concentration of $Fe^{++}+H_2O_2$, hydroxyl free radicals were detected. Exposure of the cells to this medium caused lipid peroxidation resulting in 50% inhibition of ^{14}C-deoxyglucose (DG) transport activity. Ethanol alone also inhibited DG transport in alcohol concentration dependent manner. Addition of low concentrations of ethanol increased free radical formation and hydroxyethyl free radicals were characterized. This also increased MDA formation and DG transport activity was further inhibited. Involvement of ethanol in free radical formation may provide a useful explanation for premature aging upon chronic ethanol drinking. (Supported in part by NIH grant AA02054.)

403.5

Spatial Learning Deficits in the Aged Male Rat: Neuroanatomical and Neurochemical Correlates. Lee, J.*, Lorens, S., Gower, A.*, Ross, E.* and Wulfert, E.* Dept. Pharmacol. and Pathol., Loyola Univ., Maywood, IL 60153 U.S.A., and U.C.B.,s.a., Braine-L'Alleud, Belgium

Based on their ability to locate a hidden platform in a Morris water maze, aged Sprague-Dawley rats (20-22 mo) were divided into two groups. The "old good" group acquired the task as rapidly as young (3-6 mo) animals, whereas the "old poor" rats failed to reach criterion. Using several histological methods, including antisera directed against paired helical filaments, we found no evidence for neuritic plaque or neurofibrillary tangle formation, or for abnormal cortical or hippocampal lamination in the aged animals. Compared to the young rats, both groups of old rats evidenced a reduction in the number of choline acetyltransferase (CHAT) immunoreactive cells in the ventral subdivision of the septal complex, and a decrease in CHAT cell size in the nucleus basalis. The number of CHAT labeled cells in the pontine laterodorsal tegmental nucleus in the "old poor" rats was significantly less than in the young animals. Both groups of aged rats also evidenced decreases in dopamine (DA) levels and increases in serotonin turnover in several forebrain areas. Only the "old poor" rats showed a significant decrease in medial frontal cortical DA levels. Although aged rats evidence several neuroanatomical and neurochemical changes, few are correlated with age-related deficits in spatial learning.

403.6

GLIAL FIBRILLARY ACIDIC PROTEIN mRNA INCREASES WITH AGE IN MOUSE CORTEX. J.R.Goss*, D.G.Morgan, and C.E.Finch. (SPON: S.Hess). Andrus Gerontol. Ctr. & Dept. of Biol. Sci., Univ. of Southern Calif., Los Angeles, CA 90089-0191.

Previously, we detected a 3 fold age-related increase in GFAP RNA concentration in 3 mouse brain regions by RNA gel-blot (Northern) hybridization analysis with a small number of samples. Because of our concerns that blot-hybridization analyses are at best semi-quantitative, we have performed solution hybridization (RNase protection-titration) analyses on a more extensive series of cerebral cortical samples. Male C57BL/6J mice in 4 age groups were examined for the expression of glial fibrillary acidic protein (GFAP), glutamine synthetase (GS), THY-1 antigen and beta-tubulin RNA's.

Total RNA was prepared from four cortical pools of each age group by the guanidinium thiocyanate/CsCl centrifugation method. Solution hybridization was carried out in triplicate using six concentrations of total RNA per sample. The mass of each specific message per mass of total RNA was calculated (pg/ug).

	4mo	9-10mo	17mo	26mo
GFAP	2.13±0.20	1.89±0.47	2.29±0.34	3.90±0.16
GS	13.02±1.60	14.54±0.97	13.63±1.50	14.06±1.07
THY-1	6.09±0.42	6.11±0.25	5.92±0.77	6.10±0.35
BETA-TUB	7.40±1.10	7.98±1.50	6.87±0.88	6.40±0.73

An 80% increase in GFAP message between 4 to 26 mo is the only significant change observed. This confirms our earlier limited Northern blot analysis.

An increase in GFAP without a concomitant increase in GS, both astrocyte specific RNA's, would suggest an increase in the fibrous character of astrocytes, but not in the total number of all astrocytes (fibrous plus protoplasmic). We suggest that with age there is a shift from protoplasmic to fibrous astrocytes. Future studies will compare chemical and molecular markers for the two types of astrocytes and neurons. Supported by the John Douglas French Foundation and The Anna Greenwall Award (DGM) and the A.D.R.C. of Southern California (AG-05142 to CEF).

403.7

THE EFFECT OF ESTROGEN-INDUCED ACYCLICITY ON ESTRADIOL RECEPTOR DENSITY IN THE FEMALE C57Bl/6J MOUSE BRAIN. S.G. Kohama* and C.E. Finch (SPON: P. Gray) Andrus Gerontology Center & the Dept. of Biology, University of Southern California, Los Angeles, CA 90089-0191.

Age-related declines in the reproductive neuroendocrine axis coincide with the loss of estradiol receptors (E2r) in the rodent brain (Wise and Camp, Endo 1984). A possible mechanism may involve a detrimental impact of steroids on CNS control centers, a phenomenon that may be accelerated by exogenously administered estrogens (Finch et al., Endo Rev 1984). We examined the influence of E2-induced acyclicity in young female C57Bl/6J mice on E2r populations in the brain. Mice (4.5 mo, n=4/gp) were injected sc with oil vehicle or estradiol valerate (EV). EV-treated mice remained acyclic for 5 mo and were then ovariectomized along with oil controls. After a 2 week recovery period all mice were injected ip with [2,4,6,7,16,17-^{3}H] E2, 0.05ug/10 g body weight, then were killed after 1 h. Brains were frozen, sectioned coronally and mounted on slides, then freeze-dried and apposed to tritium-sensitive film for 5 mo, then developed.

The autoradiographs were grouped by brain regions, magnified 15.6X and optical density (OD) was evaluated using the ImageMeasure software system. Controls for the labelled steroid, 100X excess cold E2, produced no exposure images. Brain regions that were analyzed encompassed the preoptic nucleus rostrally and continued caudally to the level of the mammillary nucleus. Preliminary results showed the anterior hypothalamic region of EV-treated mice to be lower in total OD versus control animals ($p<0.05$). No differences were found at the level of the preoptic area or in the more caudal regions of the hypothalamus.

This study was supported by NIA Training Grant T32-AG00093 and AG00117-10.

403.8

"ACCELERATED AGING" OF STRIATAL MUSCARINIC AUTORECEPTORS INDUCED BY ^{56}Fe IRRADIATION: IMPLICATIONS FOR FREE RADICAL INVOLVEMENT IN MEMBRANE ALTERATIONS IN SENESCENCE. J.A. JOSEPH, W.A. HUNT,* and T.K. DALTON* Gerontology Res. Ctr./NIA, Baltimore, MD 21224; Armd. Forces Radiobiol. Res. Inst., Bethesda, MD 20814.

Research has indicated that muscarinic enhancement of K^+-evoked release of endogenous dopamine (DA) from striatal slices is blunted in old animals and is correlated with deficits in motor performance. Subsequent research has indicated that the deficit may be the result of age-related membrane alterations in the muscarinic heteroreceptor (mHTR). The mechanism of this damage may occur through lipid peroxidation by free radicals. Thus, it was thought that it might be possible to mimic this damage using radiation. K^+-evoked release of DA was examined in striatal tissue slices obtained from young animals irradiated with 0-5 Gy of ^{56}Fe, and pre-tested on a wire suspension task (WST) (3-14 days post-irr.). Slices were superfused with a modified Krebs-Ringer medium containing 2.5 mM KCl. Later the medium was switched to one containing 30 mM KCl and 0 or 500 µM oxotremorine (OXO). Results indicated that both the OXO enhancement of K^+-evoked DA release and WST performance were lowered in all radiation groups at all times by 50 to 60%, suggesting that the mHTR membrane alterations that occur during aging may involve damage by free radicals.

403.9

THE NEURAL INFLUENCE ON FIBER TYPE TRANSFORMATION IN FAST-TWITCH MUSCLE DURING AGING. W.B. Alshuaib, K.S. Saiki* and M.A. Rehim. Andrus Gerontology Center, Univ. of Southern California, LA, CA 90089-0191.

The effective field of neural input on the extensor digitorum longus (EDL) was reduced by either partial ablation (ABL) of the muscle or by crushing the nerves (CN) of direct innervation in young and old male C57BL/6J mice, respectively. Sham operations were performed in the contralateral hindlimb as controls. Five weeks post-surgery, the muscle was surgically removed and tested for dynamic properties of contraction and relaxation. Subsequently, the tissue was then analyzed histochemically for transformational changes by ATP-ase and succinate dehydrogenase (SDH) staining. Dynamic and histochemical changes were compared with the contralateral limb. Peak tension in old-ABL and in old- CN muscle showed an attenuation of contraction. Muscle from the young mice also displayed a reduction of peak tension though of less magnitude. Further, half-relaxation time increased in old- ABL and old-CN. However, the half-relaxation time in young-ABL increased while it did not change significantly in young-CN. The number of slow-twitch fibers in old-ABL increased by 27±6% and in old-CN by 14±5%, whereas in young-ABL and young-CN, they increased by 44±7% and 26±8%, respectively. SDH staining showed a 31±5% increase in old-ABL and 18±5% increase in old-CN while young-ABL increased by 54±6% and young-CN by 32±6%. Although age-related differences in the magnitude of oxidative capacity were observed, as determined by SDH staining, the significantly greater change in oxidative capacity of ABL- and CN-EDL in either young or old mice when compared to histochemical ATP-ase transformation indicates an augmented adaptative response in oxidative enzyme activity.

403.10

ENDOGENOUS GLUTAMATE RELEASE FROM FRONTAL CORTEX SLICES OF ADULT AND AGED RATS. M.J. Meldrum, D.R. Wallace, and R. Dawson Jr. Dept. of Pharmacodynamics, College of Pharmacy, Univ. of Florida, Gainesville, FL 32610.

Endogenous excitatory amino acids have been implicated in several neurodegenerative disease states. The present studies were undertaken to determine whether basal and/or stimulated amino acid release was altered in adult or aged animals.

Frontal cortex was rapidly removed and cut into 600 micron coronal slices. Brain slices (4-5) were placed in nylon baskets and transferred to glass vials containing 1 ml of oxygenated Krebs Ringer bicarbonate buffer pH 7.4 at 37°C. After a 15 minute preincubation, tissues were transferred to fresh buffer. Amino acid release was stimulated by placement of slices in 56mM KCl. Amino acid release was measured by HPLC after OPA derivitization. KCl significantly increased calcium sensitive release of glutamate but not aspartate, glycine, or taurine. Basal and stimulated glutamate release were not different between 6 and 24 month old Fischer 344 rats (n = 7-8 per group). [^{3}H] glutamate uptake was also not different in frontal cortex slices of 6 and 24 month old Fischer 344 rats. These data suggest that endogenous glutamate release from slices of frontal cortex are not significantly altered in aged Fischer 344 rats. (Supported by a grant from the American Federation for Aging Research)

403.11

LEU-ENKEPHALIN (ENK) INHIBITS IN VITRO K$^+$-STIMULATED ENDOGENOUS DOPAMINE(DA) RELEASE FROM THE CAUDATE NUCLEUS(CN) OF YOUNG BUT NOT OLD MALE RATS.

V.D.Ramirez, N.J.Laping, D.E.Dluzen. Dept. of Physiology, University of Illinois, Urbana IL 61801.

CN were removed from young (2-3month) and old (20-25month) rats and placed in small superfusion chambers containing either KRP buffer or buffer with 10-8M or10-6M ENK and superfused at a flow rate of 10ul/min. After 50 minutes equilibration effluent samples were collected on ice every 10 minutes. During samples 5&6 the media were replaced with similar media containing 30mM K$^+$. Samples were analyzed for DA using HPLC-EC. The areas under the K$^+$ evoked DA release curves for each group were analyzed for significant differences. In young rats ENK reduced K$^+$-evoked DA release (10^{-8}M=448±103pg/mg, 10^{-6}M=295±31pg/mg*; * p<0.05 vs control 781±207). As shown previously, under control conditions old rats release significantly less DA than young rats in response to high K$^+$ (307±46pg/mg). In aged rats ENK had no effect on DA release regardless of the dose used (10^{-8}M=360±91pg/mg, 10^{-6}M=379±136pg/mg). These data suggest an important differential role of opiates in the control of DA release from the CN among young and aged animals.

403.12

AGE-RELATED CHANGES IN A SIMPLE PEPTIDERGIC NEURONAL SYSTEM SEEM TO BE RELATED TO LETHALITY IN THE MOLLUSC LYMNAEA STAGNALIS. C. Janse, W. C. Wildering, G. J. Van er Wilt and M. van der Roest. (SPON: EUROPEAN NEUROSCIENCE ASSOCIATION) Dept. Biology, Vrije Universiteit, 1007 MC Amsterdam, The Netherlands.

Lymnaea offers a suitable system to study aging processes in the CNS (Janse C. et al., Mech. Ageing Dev.35:179, 1986 and 42: 263, 1988; Slob W. and Janse C. Mech. Ageing Dev. 42: 275, 1988). Within the CNS of Lymnaea a system exists of two giant identfiable ACTH-like peptide containing neurons (ACTH-cells). The neurons branch extensively over the CNS and are electrotonically coupled.

Recently we found that the ACTH-cells mediate adaptive (cardio-respiratory) responses of Lymnaea to environmental oxygen levels and receive aminergic inhibitory synaptic input from oxygen senstive receptors in the skin (Van der Wilt G. J. et al., in prep.). Although in the isolated CNS both cells are spontaneously active only one of them contains a pacemaker. In addition they differ in other types of synaptic input. Obviously coupling of the cells assures functional integration.

Up to 12 months the mean coupling rate of the ACTH-cells decreases (due to increase of junctional resistance, Rj) with a concomittant increase of the individual variability of Rj (Janse C. et al., 1986; Wildering W. C. et al., in prep.). In the age groups between 12 and 20 months (ca. maximum age), however, coupling rate increased and individual variability of Rj decreased again with age. Consequently, in the very old groups only animals with high coupling rate and low Rj were found.

The variability in Rj between different animals might reflect individual variability in pace of aging between animals. The extremely low variability in the oldest group, however, is the result of the total absence of animals with low coupling rate in this group. This in turn might be due to a decreased vitality of old animals with low coupling (although recovery of coupling can not be excluded). The low individual variability in the oldest group also signifies in this view the importance of coupling between the ACTH-cells in old animals.

Due to asymmetry of the ACTH-cells coupling will contribute to the integrated response of both cells to changes in environmental oxygen levels. This, together with the increasing importance of coupling between the ACTH-cells in old animals, suggests that animals of about 12 months of age with relatively badly coupled ACTH-cells are highly vulnerable to changes in environmental oxygen levels.Experiments are in progress to test this hypothesis.

403.13

Subtractive Cloning Used to Investigate Age-Related Changes in Gene Expression in Rat Brain. M.J. Blake, J. Fargnoli*, A. Fornace*˜ and N.J. Holbrook*. Lab. Molecular Genetics NIA (GRC), Baltimore, MD. 21224 and ˜Radiation Oncology Branch, NCI, Bethesda, MD.

The brain provides an advantageous model to assess the contribution of the genome to the aging process due to its large diversity of gene products and several well characterized, age-related structural and physiological alterations. To investigate changes in mRNA expression that occur with aging, subtraction hybridization cloning was used to generate a cDNA library enriched for sequences in young adult (5mo.) rat brain by subtracting with RNA derived from old (24mo.) brain. Initial screening of the library demonstrates that several clones show a 5X or greater expression in brain than in liver, kidney or lung. Regional brain localization experiments indicate several common distribution patterns with over 60% of our clones showing a predominant expression in the cerebellum and striatum. Results indicate that changes in expression with aging show high individual variation and may be localized within specific brain regions.(Support: MacArthur Foundation; NRC Res. Assoc. to MJB)

403.14

REPEATED ADMINISTRATION OF α-MSH DOES NOT ALTER THE INCREASED ANTIPYRETIC EFFECT OF α-MSH IN AGED RABBITS. L. B. Deeter*, L. W. Martin* and J. M. Lipton (SPON: R. E. Dill). Dept. of Physiol., The Univ. Tex. Southwestern Med. Ctr. at Dallas, Dallas, TX 75235.

α-MSH, an endogenous neuropeptide, is a potent antipyretic when administered centrally or peripherally. The concentration of α-MSH in the brain decreases with age, and older rabbits exhibit greater sensitivity to centrally administered α-MSH. To determine whether this increased responsiveness of older rabbits is due to a modifiable hypersensitivity to the peptide, we attempted to alter the central reaction to α-MSH in aged rabbits via repeated injections.

Rabbits were assigned to one of four groups: old female (3-5+ years), young female (<2 years), old male (3-5+ years), or young male (<2 years). The rabbits were made febrile by i.v. injection of crude interleukin-1, and 500 ng α-MSH was injected into a lateral cerebral ventricle via an indwelling cannula. For 10 days, the rabbits were given either 500 ng α-MSH or saline, then the rabbits were tested again for antipyretic response to α-MSH. Neither aged nor younger rabbits showed altered responses to the peptide after prolonged treatment. These results indicate that the hypersensitivity of aged animals to α-MSH cannot be reduced to the level seen in younger animals, and that there is no development of tolerance to repeated injections of α-MSH.

Supported by NIH GRANT AG00109 and NINCDS NS10046.

403.15

AGE-RELATED DIFFERENCES IN BEHAVIORAL AND CEREBRAL METABOLIC RESPONSES TO MCPP IN THE RAT. U. Freo*, T.T. Soncrant*, K.M. Wozniak*, D.M. Larson* and S.I. Rapoport (SPON: H. Levitan). Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

To determine the functional significance of reported losses of brain serotonin receptors with age, we measured changes in local cerebral glucose utilization (LCGU) and behavior in young and senescent rats after treatment with m-chlorophenylpiperazine (MCPP), a preferential serotonin-1b receptor agonist. LCGU was measured with the quantitative, autoradiographic [14C]deoxyglucose method in groups of 6-8 male Fischer rats, aged 3, 12, or 24 mo, at 0, 15, or 90 min after MCPP 2.5 mg/kg i.p. Rotorod performance was assessed for 2 h after MCPP in parallel experiments. Additionally, the time course of MCPP brain concentrations was determined by HPLC analysis in 3, 12, and 24 mo rats.

Peak brain MCPP levels were reached at 15 min and were similar for all ages. The brain half-life was prolonged in older animals (75, 94, and 116 min in 3, 12, and 24 mo rats); the MCPP concentration at 90 min was 1.7 times greater in 24 mo than in 3 mo rats. MCPP impaired rotorod performance maximally at 15 min at all ages. By 90 min, 3 mo animals had completely recovered, whereas 24 mo rats remained maximally affected; 12 mo animals showed partial recovery.

LCGU was reduced ($P < 0.05$) at 15 min after MCPP in 49 of 74 brain regions of 3 mo rats (mean reduction 20%), but returned to control values by 90 min. At 15 min after MCPP in 12 and 24 mo rats, fewer regions were affected (27 and 17) and mean LCGU reductions were smaller (11 and 3%). At 90 min, LCGU showed partial recovery in 12 mo rats and minimal or no return to baseline in 24 mo animals.

These results demonstrate an age-related reduction in functional responsivity of the rat brain to serotonergic stimulation that is apparent by 12 mo of age. Prolonged effects of MCPP on behavior and LCGU in older rats probably are due to age-related changes in MCPP metabolism.

403.16

MODIFICATION OF PHORBOL ESTER BINDING AND PKC ACTIVITY IN VARIOUS BRAIN AREAS OF AGED RATS. F. Battaini, R. Del Vesco*^, S. Govoni*+, C. Lopez*^, M. Trabucchi. Chair of Toxicology, II University of Rome, ^Ist. of Pharmacol. Sci. Univ. of Milan, +Dept. of Pharmacobiology, Univ. of Bari, ITALY.

[3H]-Phorbol 12,13-dibutyrate ([3H]-PDBu) binding was investigated in various brain areas of young and aged male rats. No age-related modification in kinetic parameters were observed in cortex, hippocampus and cerebellum. In hypothalamus Bmax was decreased by 39% whereas in pituitary Bmax was almost doubled in old rats. PKC activity, measured after partial purification of the enzyme, using histone III S as substrate, was reduced in soluble fractions prepared from the cortex (61% decrease) which does not display changes in [3H]-PDBu binding. The discrepancy between binding and phosphorylating activity data could be related to an age-related modification in enzyme sensitivity to the physiological activators not involving the phorbol ester binding site. The reduced availability of PKC in soluble fraction may affect those neuronal mechanisms involving a transfer (and therefore the activation) of PKC molecules from the cytoplasmic pool to membranes.

403.17

SIMILARITIES BETWEEN RADIATION AND AGING ON THE FUNCTION OF SUBSTANTIA NIGRA DOPAMINE NEURONS. J. Frascella, J.A. Joseph, & W.E. Murray* Behavioral Sciences Dept., Armed Forces Radiobiol. Res. Inst., Bethesda, MD 20814.

The effects of radiation and aging on motor function are similar (e.g. ataxia, loss of balance and coordination), and studies have revealed that changes that take place with nigrostriatal dopamine (DA) cells in aging lead to degenerative motor disorders. A proposed mechanism for the changes produced by aging is the generation of and inability to scavenge free radicals. Because ionizing radiation is very effective in generating free radicals, the purpose of this study was to investigate the functioning of nigral DA cells in radiation-exposed and aged rats in an attempt to determine a common mechanism underlying motor deficits observed with radiation and aging.

Substantia nigra DA cells in chloral hydrate anesthetized Fischer rats were recorded extracellularly by conventional techniques. Irradiated rats were exposed to 60 Gy of either γ-photons or high-energy electrons, and aged rats were between 26 and 32 months of age. Autoreceptor-specific doses (0.5-2.0 μg/kg, i.v.) of the DA agonist, apomorphine, were administered, and results showed that compared to controls, apomorphine inhibited the firing rate of DA cells to a greater extent in the irradiated and aged animals. Also, DA cells from both groups showed a greater sensitivity (increased firing rate) to the DA antagonist, haloperidol, at low doses (50-100 μg/kg, i.v.). Thus, these results suggest that a common mechanism might produce the changes in DA cell function in both irradiated and aged animals. This mechanism could be related to increased amounts of free radicals present in the nigrostriatal pathway that would affect nigral cell function and consequently motor behavior.

403.18

Pathlength analysis of age-related dendritic regression. R. Pentney, H. Ngo, and L. Quackenbush, Dept. of Anat. Sci., State Univ. of NY, Buffalo, NY 14214

A new method for analysis of dendritic regression in the Purkinje cell (PC) network showed that age-related PC regression was confined to terminal dendritic links (TL) and that it progressed by gradual shortening of TL rather than by deletion of TL at junctions (Pentney et al, Anat. Rec. 220:79A, 1988). Subsequent application of the method to PC networks from alcoholic rats (Pentney and Quackenbush, Alcoholism: Clin and Exper Res. 12:324, 1988) suggested a need for precise localization of affected network terminals by measurement of dendritic pathlengths (dendritic lengths between soma and TL tips). The method developed and applied here to networks from aging rats will be applied subsequently to networks from aging, alcoholic cohorts. Cerebellar cortices from Fischer 344 rats, 10, 18, and 28 months of age, were prepared by the Golgi-Cox procedure. Randomly selected PC networks from coded parasagittal sections were drawn and then measured on a digitizing tablet. Pathlengths for all terminals were obtained by computer analysis of these measurements, then averaged and sorted for minimum and maximum values. The data revealed that the maximum dendritic pathlength decreased significantly in PC networks of 18 month old rats ($p<.05$). Ongoing analysis of frequency histograms of pathlengths will provide data reflecting changes in density of terminals within networks. (NIAAA grant AA 05592)

BEHAVIORAL PHARMACOLOGY: MONOAMINES

404.1

SOUND PRESSURE LEVEL AND ADRENOCEPTOR INFLUENCES ON AUDIOGENIC SEIZURES IN DBA AND PRIMED C57BL MICE. C.E. Lints, A. Mayall* and D. Capruso*. Dept. of Psychology, Northern Ill. Univ., DeKalb, IL 60115.

Experiment 1 examined the relationship between sound intensity and audiogenic seizure (AGS) activity in 21- and 30-day-old genetically seizure prone DBA mice. At both ages, and for the intensities tested, AGS activity increased as sound intensity was increased above seizure threshold. However, the higher intensities were less effective in eliciting the full AGS syndrome in the older mice. In Experiment 2, the alpha-1 antagonist prazosin produced anticonvulsant effects in 21-day-old DBA mice. The alpha-2 antagonist yohimbine failed to exert proconvulsant effects at the doses tested, although the higher doses produced anticonvulsant effects. In Experiments 3 and 4 genetically seizure resistant C57BL mice were first rendered AGS susceptible through acoustic priming at 16 days-of-age and then tested as in Experiments 1 and 2 when they were 21 days old, replicating the results with DBA mice. In addition, the beta-1 antagonist atenolol produced anticonvulsant effects in the primed mice (Experiment 4). The results suggest that primed AGS in C57BL mice may represent a neuropharmacological phenocopy of the DBA syndrome, and that the midbrain adrenoceptor hypothesis may extend to primed C57BL mice.

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404.2

FOREBRAIN NOREPINEPHRINE (NE) LESIONS DO NOT ELIMINATE THE EFFECTS OF ENRICHED ENVIRONMENTS ON SPATIAL MAZE PERFORMANCE. Susan J.E. Murtha, Bruce A. Pappas, and Shankar Raman*. Dept. of Psychology, Carleton Univ., Ottawa, Ont., K1S 5B6.

Previous research has shown that neonatal systemic 6-hydroxydopamine (6-OHDA) eliminates several effects of enriched (E) vs impoverished (I) rearing. Since this treatment not only lesions forebrain NE terminals but also causes a peripheral sympathectomy, either of these effects or their combination could alter the response to E/I rearing. Accordingly, we examined the effects of lesioning only the brain by intraventricular 6-OHDA in the neonate or 6-OHDA lesion of the dorsal NE bundle in the adult.

At 24 and 48 hours after birth, buproprion pretreated (to protect dopamine terminals) rats received bilateral intraventricular injection of 12.5 ug 6-OHDA. At weaning, half the rats were raised in E or I environments respectively for 35 days. The adult treated rats received 6-OHDA (4 ug) into the dorsal tegmental bundle and were subsequently housed in E or I conditions for 42 days. Following this the rats were extensively tested in a modified Hebb-Williams maze. Both the infant and the adult E-housed control rats made fewer errors than their I-housed counterparts. Furthermore, although both the neonatal and the adult lesioned rats showed substantial and selective lesions of forebrain NE terminals, these rats also showed a performance enhancing effect of E rearing. We conclude that lesioning of forebrain NE by itself does not eliminate the beneficial effects of chronic exposure to an E environment on spatial problem solving.

404.3

EFFECTS OF CHRONIC CENTRAL VERSUS PERIPHERAL INFUSION OF A BETA-BLOCKER ON AGGRESSION. M.L. Leavitt*, J.C. Maroon*, S.C. Yudofsky*, E.J. Riley*, and M.B. Bavitz*. (SPON: W.E. Hoffman). Allegheny-Singer Research Institute, Pittsburgh, PA 15212-9986.

The lipophobic beta blocker nadolol (Nad) clinically decreases aggressive behavior following large daily oral doses. To investigate its mode of action, we have compared the effects of intraventricular (IVT) versus subcutaneous (SQ) infusion of Nad on shock-induced attack behavior (SIA). Male 6-hydroxydopamine-treated rats (IVT, 2x200 µg) had baseline SIA measured before implanting minipumps that infused either: A. IVT Nad (0.25 mg/kg/day, N=8); B. IVT Nad (0.01 mg/kg/day, N=5); C. SQ Nad (0.25 mg/kg/day, N=5); D. SQ Nad (2.5 mg/kg/day, N=6); or E. SQ saline-mock pumps (N=5). SIA was monitored every 2-3 days and averaged over consecutive 7-day periods. SIA was significantly reduced compared to baseline during IVT Nad infusion at both 0.25 and 0.01 mg/kg/day. SQ Nad also reduced SIA but only at a dose 10 times the highest IVT dose.

	Baseline	Week 1	Week 2	Week 3	Week 4
A	41.6±1.4	32.2±1.6§	30.2±1.5§	-	-
B	41.5±2.2	34.1±2.6*	31.4±2.4*	-	-
C	36.7±1.0	36.2±0.9	37.7±1.4	-	-
D	40.2±2.3	30.8±3.8*	33.4±4.2	29.6±3.8*	27.2±2.6^{+}
E	37.4±4.0	35.9±4.8	37.2±2.6	39.1±0.9	40.8±2.2

*p<.05, $^{+}$p<.005, §p<.001 vs. baseline

These results suggest that lipophobic beta blockers may reduce aggressive behavior via a central mechanism.

404.4

EXTENDED BLOCKADE OF QUIPAZINE CUE CORRELATES WITH CHANGE IN 5HT-2 RECEPTOR SENSITIVITY, BUT NOT BINDING. R.L. Smith, R.J. Barrett, and E. Sanders-Bush. Dept. of Psychology and Pharmacology, Vanderbilt Univ. and VA Medical Center, Nashville, TN 37209

The present study was conducted to investigate the duration of action of 5HT-2 antagonists. Rats were trained on a saline-quipazine (Q) (1.5 mg/kg) discrimination. Following acquisition, independent groups were injected with 2 mg/kg mianserin (M). These groups were then tested at 12 h intervals for their ability to discriminate Q. Blockade of the Q cue persisted for up to 60 h following M administration. Duration of blockade was dose-dependent. Two other 5HT-2 antagonists, pizotifen and metergoline, showed similar profiles of extended antagonism. Changes in 5HT-2 receptor density and sensitivity were investigated as possible mechanisms for M's prolonged antagonism. M (2 mg/kg) caused a decrease (52%) in the density of 5HT-2 sites at 24 h but was recovered at 36 h. 5HT-2 stimulated phosphoinositide hydrolysis was used to assess 5HT-2 receptor sensitivity. Mianserin caused a significant reduction in the maximum response that persisted for 60 h. Hence, the time course for recovery of receptor sensitivity corresponded to that of the behavior while receptor density did not. (Supported by MH34007 and Veterans' Administration.)

404.5

PHARMACOLOGICAL EVALUATION OF 5-HT$_1$ RECEPTOR MEDIATED TURNING AND BEHAVIORAL SYNDROME IN THE RAT.
E.Horváth*, J.De Vry*, T.Glaser* and J.Traber* (SPON: F.K. Pierau), Neurobiology Department, Tropon Pharmaceuticals, Neurather Ring 1, D-5000 Köln 80, F.R.G.

A substantial body of evidence points to an important role of central 5-HT receptors in various psychological processes. To study those receptors and selective ligands in vivo, suitable animal models have to be pharmacologically characterized. In rats with an unilateral lesion of the dorsal raphe nucleus potent 5-HT$_{1A}$ receptor agonists such as 8-OH-DPAT (Blackburn et al., Psychopharmacol. 83: 163-165, 1984) or BAY R 1531 induced dose-dependently contralateral turning. This behavior could be prevented by pretreatment with the non-selective 5-HT antagonist methiothepine, and partially by racemic pindolol; whereas the 5-HT$_2$ receptor antagonist ritanserin was inactive. Neither of the 5-HT antagonists induced contra- or ipsilateral turning themselves. Compounds acting on other subtypes of 5-HT receptors like TFMPP, ICS 205-930 and GR38032F were inactive when applied alone. DA agonists such as SKF 38393 and pergolide were ineffective and the DA antagonists SCH 23390 and haloperidol failed to inhibit 8-OH-DPAT induced turning. The 5-HT$_{1A}$ receptor partial agonists ipsapirone, buspirone and gepirone partially induced turning and partially blocked 8-OH-DPAT activity. In intact rats, 5-HT$_{1A}$ receptor agonists reliably induced forepaw treading, Straub's tail and locomotor activity in a dose-dependent manner. 5-HT$_{1A}$ receptor partial agonists as well as (non)selective ligands for the other 5-HT receptor subtypes failed to induce these elements of the 5-HT syndrome. These data show strong quantitative and qualitative similarities with those obtained in the turning model and also correlate well with in vitro data.

404.6

HABITUATION AND DIAZEPAM SUPPRESS NOVELTY-INDUCED ACTIVITY IN ASCENDING 5-HT SYSTEMS. S.R. Bodnoff, D.H. Aitken, M. Doherty, B.E. Suranyi-Cadotte, R. Quirion, and M.J. Meaney. Douglas Hospital Research Ctr., Dept. Psychiatry McGill Univ., Montréal H4H 1R3, Canada.

Novelty results in increased arousal and a suppression of appetitive behaviors in the rat. Repeated exposure to an innocous, novel environment (habituation learning), alleviates these responses and the effectiveness of these stimuli to inhibit appetitive behaviors. This process can be circumvented with anxiolytics: Hungry animals given food in a novel environmenteat more readily following benzodiazepine (BZ) treatment, mimicking the effects of habituation. Likewise, RO15-1788 (central BZ receptor antagonist) blocks the behavioral effects of habituation.

We then exposed food-deprived rats to food in a novel environment following 0 or 7 days of habituation, or an injection of diazepam (2 mg/kg). Both 7-days habituation and diazepam decreased the latency to begin feeding. All animals were sacrificed 5 min following exposure to the novel environment and 300 uM brain sections were processed for microdissection. Norepinepherine (NE), serotonin (5-HT), and their metabolites were measured using HPLC with electro-chemical detection. In the raphé nuclei, both habituation and diazepam decreased 5-HT content and turnover and decreased NE content compared to controls. Thus, habituation and BZ's appear to share a common mechanism of action that inhibits ascending 5-HT systems and anxiety.

404.7

5HT$_{1A}$ AGONISTS AND EXCITATORY AMINO ACID ANTAGONISTS EXHIBIT ANXIOLYTIC ACTIVITY IN THE SOCIAL INTERACTION TEST AND THE ELEVATED PLUS MAZE. R. Corbett*, R. W. Dunn*, H. M. Geyer III*, M. Cornfeldt and S. Fielding. (Spon: C. P. Smith). Department of Biological Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

In the social interaction (SI) test, anxiety is generated by the novel situation of placing unfamiliar pairs of rats together in a familar setting, while in the elevated plus maze, exposure to an elevated open alley results in an approach-avoidance conflict in rats. In the present study, two classes of compounds, namely, the 5HT$_{1A}$ agonists and NMDA antagonists, showed anxiolytic activity in these paradigms. Following intraperitoneal administration, the 5HT$_{1A}$ agonists 8-OH-DPAT (0.2-0.4 mg/kg), buspirone (1-10 mg/kg) and gepirone (2.5-10 mg/kg) significantly increased SI time by +114%, +69% and +78%, respectively, while increasing open alley exploration (AE) by +108%, 70% and 43%. Likewise, the N-methyl-D-aspartate (NMDA) antagonists, CPP (2.5-10 mg/kg), 2-amino-5-phosphonopentanoic acid (30-60 mg/kg) 2-amino-7-phosphonoheptanoic acid (20-40 mg/kg) and the noncompetitive NMDA antagonist, MK-801 (0.05-0.1 mg/kg) increased SI by +59%, +73%, +116% and +51%, respectively, while increasing AE by +88%, +92%, +30% and +88%. In summary, both 5HT$_{1A}$ agonists and NMDA antagonists were anxiolytic in two different anxiety tests, suggesting that anxiety is mediated by serotonin and excitatory amino acid systems.

404.8

ULTRASONIC VOCALISATIONS BY RAT PUPS AS AN ANIMAL MODEL FOR ANXIOLYTIC ACTIVITY. J.Mos*, P.Bevan and B.Olivier. Dept of Pharmacology, Duphar B.V., P.O.Box 2, 1380 AA Weesp, The Netherlands.

Rat pups separated from their mother and littermates produce ultrasonic calls with a frequency of 40-55 kHz. Pups were tested at an age between 9 and 12 days during a 5 min period. Ultrasonic recording equipment and counters were used to measure the effects of antidepressants under two environmental conditions, viz. a cold (18°C) and a warm (37°C) plate. The more stressful cold plate evoked considerably more calls than the warm plate.

Benzodiazepines reduced ultrasounds under both conditions thereby confirming their anxiolytic activity. The purported 5-HT$_{1a}$ ligands 8-OH-DPAT, buspirone and ipsapirone all reduced ultrasounds under both conditions. The mixed 5-HT$_{1a/b}$ agonists RU24969 and eltoprazine (DU 28853) reduced ultrasonic calling only under the cold plate condition. The same applied for the 5-HT$_{1b}$ agonist TFMPP and the mixed 5-HT$_{1/2}$ agonist quipazine. Antagonists of the 5-HT receptor -methysergide and ritanserine- were ineffective in suppressing the ultrasonic calls. Drugs affecting serotonin turnover by inhibiting reuptake, such as fluvoxamine and zimeldine reduced pup ultrasounds when tested under cold testing conditions.

As reference compounds, pentobarbital, scopolamine, d-amphetamine, pentrazol, sulpiride and haloperidol were tested, none of which affected ultrasound production in a dose-dependent way.

In this animal model, non benzodiazepine anxiolytics (5-HT$_{1a}$ ligands) are thus reliably detected. At present we view those substances that only inhibit the pups reactions at the cold plate as potentially anxiolytic, albeit weaker than the benzodiazepines and the more recently developed non-benzodiazepine anxiolytics.

404.9

NORFENFLURAMINE PROVIDES STIMULUS CONTROL: COMPARISON WITH FENFLURAMINE. D. McBurney*, J.W. Boja, and M.D. Schechter, Dept. Pharmacol. Northeastern Ohio Univ. Coll. of Med., Rootstown, OH 44272.

Male rats were trained to discriminate 1.4 mg/kg norfenfluramine (NF) from its vehicle using a two-lever, food-motivated operant discrimination task. Once trained, the rats showed a dose-dependent decrease in responding on the NF-correct lever following decreased doses of NF (ED_{50}=0.71 mg/kg). Administration of 2.0 mg/kg fenfluramine (FEN) produced 100% responding on the NF-correct lever and decreasing doses of FEN, likewise, produced a dose-dependent decrease in responding on the NF-correct lever (ED_{50}=1.30 mg/kg). Time-course data indicated that NF has a fast onset and a peak effect at 20-60 min after administration. Analysis of the time-course data provided a half-life of approximately 8 hrs. In contrast, FEN did not show the rapid onset that was observed with NF. However, FEN had a similar peak effect and half-life. These results indicate a pharmacological similarity between NF and FEN, However, the difference in onset of action suggests a possible difference between the parent drug and its metabolite.

404.10

SUBSENSITIVITY TO THE BEHAVIORAL EFFECTS OF THE TRH ANALOG MK-771 FOLLOWING TREATMENT WITH 3-ACETYLPYRIDINE (3-AP). M.S. Kreider, S. Wieland and I. Lucki.(SPON: J. Winkler). Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA 19104.

Systemic administration of 3-AP to rats produces lesions of the inferior olivary complex resulting in dystonic symptoms. We studied the functional and morphological integrity of the TRH system in rats treated with 3-AP by examining the following: head shake response produced by the TRH agonist MK-771, TRH levels in 12 brain regions, and the density of TRH receptors using receptor autoradiography.

Treatment with 3-AP significantly reduced the ability of MK-771 (0.63-10.0 mg/kg) to produce the head shake response. The ED50 value for MK-771 was shifted to the right and the peak response was reduced by 50% after 3-AP. TRH levels were unchanged in all brain regions following 3-AP treatment. TRH receptor density of measured using ^{3}H-MeTRH, was significantly reduced in laminae 4-10 of the cervical spinal cord. The subsensitivity found to the behavioral effects of MK-771 following 3-AP treatment may be associated with a down-regulation of TRH receptors.

This research has been supported by the Dystonia Medical Research Foundation and USPHS Grant GM 34781.

404.11

ENHANCED SENSITIVITY TO THE BEHAVIORAL EFFECTS OF SEROTONIN (5-HT) AGONISTS FOLLOWING TREATMENT WITH 3-ACETYLPYRIDINE (3-AP). S. Wieland, P. McGonigle and I. Lucki. Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA 19104.

Systemic administration of 3-AP to rats produces lesions of the inferior olivary complex resulting in symptoms of dystonia. We studied the functional and morphological integrity of the 5-HT system in rats treated with 3-AP by examining the following: two behavioral responses caused by stimulating 5-HT receptors, 5-HT and 5-HIAA content in six different brain regions, and the density of 5-HT_{1A} and 5-HT_2 receptors using receptor autoradiography.

Rats treated with 3-AP were 3-fold more sensitive to the ability of the 5-HT_{1A} agonist 8-OHDPAT to produce the 5-HT syndrome. Similarly, the 3-AP treated rats were 2-fold more sensitive to the selective 5-HT_2 agonist (+)-DOB at causing the head shake response. 5-HT and 5-HIAA levels were unchanged in six brain regions following 3-AP treatment. 5-HT_{1A} receptor density measured using ^{3}H-8-OHDPAT, was unchanged in the spinal cord, the CNS area associated with the 5-HT syndrome. Presently, the supersensitivity found to the behavioral effects of 5-HT agonists following 3-AP treatment cannot be explained by direct morphological changes to 5-HT neurons or receptors.

This research has been supported by the Dystonia Medical Research Foundation and USPHS Grant GM 34781.

HUMAN BEHAVIORAL NEUROBIOLOGY III

405.1

REGIONAL CORTICAL BLOOD FLOW DURING COGNITIVE ACTIVATION IN DOWN SYNDROME. K. F. Berman, M. B. Schapiro*, R. P. Friedland, S. I. Rapoport and D. R. Weinberger. Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032, and Laboratory of Neurosciences, NIA, NIH, Bethesda, MD 20892.

Few studies of pathophysiology in young, cognitively disabled patients, such as those with Down Syndrome (DS), have been carried out. Using the xenon133 inhalation method, we measured regional cortical blood flow (rCBF) in 11 non-institutionalized physically healthy, mildly to moderately retarded trisomy 21 DS patients (three women and eight men; mean age = 28 years; mean mental age on Peabody Picture Vocabulary = Test 8 years, range 4 to 16) and 22 age- and sex-matched normal controls. Following an initial resting state rCBF study on each of two consecutive days, subjects had rCBF measured during four different cognitive activation conditions: on day 1, during Raven's Progressive Matrices (RPM), which is a posterior cortical activator, and during a nonspecific control task; and on day 2, during the prefrontally-linked Wisconsin Card Sort (WCS) and during another nonspecific control task.

Despite poor performance on the WCS, DS patients increased prefrontal rCBF during the WCS compared to baseline (mean [$\pm$ SEM] change in rCBF, 5.0 $\pm$ 1.7 IS units, $P < .02$, paired T-test) in a fashion similar to normals (4.6 $\pm$ 1.9, $p < .03$). During RPM there were no significant differences between the two groups, but DS patients did not activate posterior cortical areas as normals did (patients: 0.9 $\pm$ 1.6, $p = .57$; normals: 4.7 $\pm$ 1.4, $p < .004$). These data may suggest differential pathophysiological involvement of anterior and posterior cortical systems in DS.

405.2

NEUROMAGNETIC VISUAL EVOKED RESPONSES TO SINUSOIDAL GRATINGS. J. S. George*, C. J. Aine, and E. R. Flynn, (SPON: D. L. Arthur). Neuromagnetism Laboratory, Los Alamos National Laboratory, MS M882, Los Alamos, NM 87545.

Neuromagnetic evoked responses were recorded for visual stimuli presented in the central visual field (CVF) or 8° in the right visual field (RVF). Stimuli were high contrast, intensity-modulated vertical sinusoidal gratings of 1 (Lo) or 5 (Hi) cycles per degree, that occupied 2.0°H x 1.5°V. Neuromagnetic data were collected at 42 sensor locations over occipital and parietal regions. Field amplitudes were sampled at 10 ms intervals from continuous waveform data to produce a temporal series of neuromagnetic field maps which were then fit by a current dipole model using least squares procedures. In this presentation we focus on data from three human subjects. Calculated RVF response sources were deeper than those for corresponding CVF stimuli. Field amplitudes and calculated current moment for the initial response peak were smaller for RVF than for CVF stimuli and generally peaked earlier. Major differences in field distribution and calculated source location and orientation were observed as a function of visual field of stimulation. Smaller but apparently significant differences were observed as a function of spatial frequency in early response components for a particular stimulus location. Lo spatial frequency (SF) gratings produced larger field amplitudes and larger current moments than Hi SF for RVF stimuli.

405.3

FRONTAL EEG COHERENCE AND INTELLIGENCE--A POSITIVE CORRELATION. R.S. Hernandez, A.T. Arenander, W.D. Sheppard, R.W. Boyer, and M.C. Dillbeck. Depts. of Physiological and Biological Sciences, and Psychology, Maharishi International University, Fairfield, IA 52556.

The relation of cortical EEG coherence (COH) and measures of cognitive performance have been reported by a number of researchers. In particular, Thatcher et al. (Cognitive Processing in the Right Hemisphere, Perecman, '83, 125-146) found a negative correlation between COH during eyes-closed (EC) rest and IQ in children. In contrast, Orme-Johnson et al. (abst. 15th Winter Conf. on Brain Res.) found a positive correlation between frontal COH during Transcendental Meditation (TM) practice and IQ in adults. This study attempted to reconcile these findings and to further clarify the functional significance of cortical COH by testing COH-IQ correlations across a dynamic range of mental states. We report here an anterior-posterior gradient in the COH-IQ correlations (corr.) in children across three task conditions tested.

The WISC-R was administered to 48 children (10 to 16 years). EEG COH was calculated for 4 frequency bands across 16 scalp leads during 3 task conditions: EC rest, a mental arithmetic task, and TM practice. Polynomial regression analyses of full scale IQ regressed on each of the COH variables demonstrated significant negative corr. in posterior scalp locations across all frequency bands and all task conditions. However, a consistent trend toward positive corr. was found in frontal leads for all 4 frequency bands in all task conditons. Multiple analyses of covariance demonstrated that the differences in anterior and posterior corr. were significant in delta and theta frequencies in all task conditions. The negative posterior COH-IQ correlations reported here across task conditions indicate the robustness of previous findings. In contrast, the significant postitive frontal corr. suggest that the functional significance of COH may depend upon scalp location, with frontal COH being distinct from posterior regions. These findings also suggest that TM practice produces measurable changes in the functional activity of frontal areas of the brain.

405.4

EEG COHERENCE AND SEMANTIC PROCESSING UNDER SUBLIMINAL AND SUPRALIMINAL TASK CONDITIONS. W.D. Sheppard II and R.W. Boyer.* Dept. of Biological and Physiological Sciences, Maharishi International University, Fairfield, IA 52556.

Twenty-four subjects were presented 240 randomized trials of a Lexical Decision Task (LDT) while connected to a 16 electrode EEG montage. EEG data were acquired for two seconds prior to the onset of each LDT trial. Each LDT trial consisted of a fixation asterisk (6-12s), a prime letter string (17 or 153ms), a pattern mask (300ms), and a target letter string which remained on the monitor until the subject pressed one of two buttons, word or nonword. Following the lexical decision the subject gave a verbal report identifying the prime as word or neutral and a number estimate of the confidence of that report. Results showed significantly different relationships between coherence and semantic effects depending upon prime duration and prime-target relatedness. High pretrial coherence in left frontal areas was associated with more efficient processing in fast prime trials but less efficient processing in slow prime trials. High coherence in left temporal-parietal areas showed the opposite trend but only in related prime-target trials. The relationship between high interhemispheric coherence and semantic effect was significantly different in fast vs slow prime trials but only in the unrelated prime-target trials. A model relating flow of information during cognitive processing and coherence is discussed.

405.5

DIFFERENTIAL PROCESSING OF AUDITORY STIMULI CONTINUES DURING SLEEP Nielsen-Bohlman*, L., Knight, R. T., Woods, D. L., & Woodward*, K. Neurology Dept., U. C., Davis, V.A.M.C., 150 Muir Rd., Martinez, Ca. 94553.

The principal component of the waking auditory evoked potential (AEP) is the N1, a negative potential peaking at 100 msec. We examined N1 amplitude in waking and stage II-IV sleep, and compared it to the most prominent sleep AEP, the N2 (latency 300 msec). Eighty percent of stimuli were 1.0KHz tones (1.0 sec ISI, 60dBSL). Response to stimulus deviance was evaluated by randomly presented tones of a different frequency (1.5KHz) or complex novel sounds, each occurring in 10% of trials. The waking N1 was maximal in amplitude at frontal scalp sites, with small additional peaks at temporal sites. Deviant stimuli generated a mismatch negativity (MMN) of 4.0 uV at frontal sites. During stage II sleep N1 amplitude was reduced. MMN was present but also reduced to 1.0 uV. In stage III-IV sleep N1 was abolished at all sites. AEPs during stage II-IV sleep were dominated by a centrally distributed N2 component (latency 324 msec). N2 amplitude increased in response to deviant stimuli in all sleep stages. These data suggest that the intracranial sources of AEPs change from wakefulness to sleep. Furthermore, the augmented response to deviant sounds observed during waking and all sleep stages indicates that differential processing of auditory stimuli persists during sleep.

405.6

SEX DIFFERENCES IN THE ERPS TO VERBAL AND NONVERBAL MEMORY TASKS. M.J. Taylor*, M.L. Smith* and K. Iron* (SPON: W.J. Logan). Div. of Neurol., Hosp. for Sick Children and Dept. of Psychol., Univ. of Toronto, Toronto, Canada, M5G 1X8.

Recently it has been suggested that patterns of hemispheric specialization vary with gender. For example, studies of aphasic and apraxic patients indicate that men and women differ in terms of anterior to posterior organization of function within the left hemisphere (Kimura, 1983, 1987, Can. J. Psych.). In the present study, gender differences in the neurophysiological substrates underlying memory were investigated in normal subjects (13 women, 8 men) by recording the event related potentials (ERPs) from 19 scalp electrodes during performance on visual recognition tasks for recurring verbal items (RV) and recurring abstract figures (RF). The mean reaction times did not differ for the tasks, whereas the latencies of the memory-related components (N4 and P3) were significantly longer for RF. Significant lateral asymmetries were seen only in N4 (larger on the left in both tasks). No significant differences were found between the tasks or sexes in the reaction times or error rates. There were significant sex by task interactions in the ERPs due to larger ERPs anteriorly for females for both tasks, but slightly larger ERPs posteriorly for males in the RV task. This was found only for the cognitive components (P2, N4, P3). The differences found in normal subjects between the sexes extend the evidence from neuropsychological studies in patient populations that men and women differ in terms of anterior to posterior organization of function.

405.7

EVENT-RELATED POTENTIALS TO VISUAL "POP-OUT" STIMULI. S.J. Luck* & S.A. Hillyard (SPON: G.R. Mangun). Dept. of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

When a visual display contains an item that differs from the background on the basis of a basic visual feature, that item can be discriminated by an early, preattentive stage of processing. In subjective terms, it appears to "pop out" from the rest of the display. We recorded event-related brain potentials from 12 young adults while they engaged in a task that required them to discriminate pop-out stimuli.

Subjects were presented with displays of 8 randomly located colored bars. On no-pop-out trials (p=.5) all 8 bars were blue and vertical, and on pop-out trials, 1 of the bars was either horizontal (p=.17), green (p=.17), or wide (p=.17). For each run, 1 of the 3 pop-outs was the target, while the other 2 pop-out and no-pop-out displays were non-targets. Target stimuli elicited enhanced frontal P2, posterior N2, and broad P3 components. The posterior N2 was largest at scalp sites contralateral to the side of the pop-out and may represent the operation of focal attention. Both target and non-target pop-outs elicited a frontal N2 component, suggesting an automatic orienting to discrepant items regardless of their relevance.

405.8

THE CHRONOMETRY OF SELECTIVE ATTENTION AND MISMATCH DETECTION. GP NOVAK, WR RITTER, AND HG VAUGHAN*. RF Kennedy Ctr, Albert Einstein Coll of Med, Bronx, NY 10461.

In this selective attention task, targets were rare longer (170ms) tones of a designated pitch, imbedded in a sequence of 100ms standards. An additional simple reaction time (SRT) condition required a response to an unvarying standard. Event related potentials (ERPs) were recorded from 15 sites referred to the nose.

Selective attention effects were evident in the ERPs to standards. Attended standard ERPs were closely similar for easy (1000Hz vs 2000Hz) and hard (1000Hz vs 1030Hz) pitch separations, and were more negative frontocentrally than unattended standard ERPs. Difference waveforms (attended-unattended standards) revealed a negative deflection (Nd), earlier in latency for the easy task (onset, 170ms; peak, 250ms) than for the hard task (onset, 250ms; peak, 350ms). Unlike N1, Nd did not invert in polarity posterolaterally. Attentional effects for both attended and unattended standards were seen in comparison with SRT ERPs; difference waveforms (standard-SRT) revealed a negative deflection (NA), earlier in onset (50ms post stimulus onset) but identical in topography to Nd. The speed of detection of the deviant longer tones was insensitive to the attentional processes indexed by Nd. Median reaction time did not differ between tasks (easy, 510ms; hard, 513ms). Neither attention nor task difficulty affected the latency of mismatch negativity (MMN), N2 or P3 (as identified in difference waveforms: attended or unattended longer tones minus their respective standards). MMN onset and peak latencies (140ms and 185ms, respectively) preceded those of Nd, even for the easy pitch separation. Accuracy of response was determined by attention-related selection of the relevant pitch. There were more misses (28% vs 14%) and false alarms (6.7% vs 0.3%) in the hard task, and nearly all of the latter were responses to the irrelevant longer tone. The data suggest that performance is guided by two parallel and independent processes, automatic mismatch detection of the longer tone followed by selection of response based on match to the attentional template for pitch.

405.9

BRAIN POTENTIALS PREDICTIVE OF LATER PERFORMANCE ON TESTS OF RECALL, RECOGNITION, AND PRIMING. K.A. Paller, C.C. Wood, and G. McCarthy. Neuropsychology Laboratory, VA Hospital (116B1), West Haven, CT 06516 and Depts. of Neurology and Psychology, Yale Univ.

Despite the connection established between the brain areas damaged in amnesia and *declarative memory*, the functional roles of these brain areas are currently unclear. Information derived from the electrical activity generated in these brain areas during memory tasks may be useful for understanding the brain mechanisms mediating declarative memory. Event-related potentials (ERPs) may be sensitive to such activity and can be recorded from human subjects engaged in complex verbal tasks. Correlations between ERPs and later memory performance have been reported previously. In these studies, ERPs elicited by words that were later remembered were generally more positive than ERPs elicited by words that were later forgotten.

The present experiments investigated electrophysiological correlates of memory performance in relation to retention interval and type of memory test (free recall, yes-no recognition, and stem-completion priming). In the priming test (a test of *nondeclarative memory*), 3-letter stems corresponding to some of the 200 words presented earlier were mixed with an equal number of additional stems. Subjects were instructed to complete each stem verbally with the first word to come to mind. The number of completions to presented words constituted priming performance. The recall test was given after a 15-min delay, whereas recognition and priming tests were given either after a 1-min delay or after a 15-min delay.

An average of 11% of the words were recalled. The number of words recognized did not differ as a function of delay and averaged 57% (chance=14%). Priming scores were much greater with the short delay (45%) than with the long delay (18%), and in both cases were significantly greater than chance (10%). ERPs elicited during acquisition differed as a function of later memory performance. ERPs to recalled words were relatively more positive than ERPs to unrecalled words, especially 600 ms after word onset. Similar though less consistent results were obtained for recognition and priming. Preliminary results were also obtained from epileptic patients with electrodes implanted in medial temporal and other brain regions.

405.10

VISUAL SELECTIVE ATTENTION TO LINGUISTIC STIMULI: INTRACRANIAL ERP RECORDINGS. A.C. Nobre, G. McCarthy. Neuropsychology Laboratory, VA Medical Center, West Haven, CT 06516 and Depts. of Neurology and Psychology, Yale University.

We have previously demonstrated changes in scalp event-related potentials (ERPs) associated with the differential processing of concurrent visually presented stories (Nobre & McCarthy, *Soc. Neurosci. Abstr.*, 1987). The spatio-temporal characteristics of the ERP effect suggested that it is generated by multiple neural sources. Since neural generators cannot be determinded from scalp distributions alone, intracranial ERPs were obtained from a group of patients being evaluated as candidates for surgery to relieve medically intractible seizures. Each patient had chronically implanted unilateral or bilateral posterio-temporal (PT) multi-contact depth probe(s). The first several contacts of the PT probe(s) sampled activity in extrastriate visual cortex, while the deeper contacts were in the medial temporal lobe.

Words comprising two stories were randomly intermixed and displayed briefly and successively on the center of a computer display, with words in each story displayed in a single color (red or green). Subjects were instructed to read silently either the red or green story. Comprehension of the attended story was tested immediately at the end of each run.

Like the ERPs recorded from the scalp, the intracranial ERPs recorded from the PT probes' superficial contacts showed a large attentional effect in four out of five patients. The morphology and time-course of the effect were strikingly similar to that recorded from the occipital (O_z) scalp electrode in normal subjects. The intracranial effect, however, was approximately an order of magnitude larger in amplitude and opposite in polarity from that observed in its scalp counterpart. This polarity inversion suggests that one generator of the selective attention effect recorded at the scalp may be located in the extrastriate occipital cortex.

405.11

THE EFFECTS OF SPEED-ACCURACY INSTRUCTIONS ON REACTION TIME AND P3 LATENCY IN STIMULUS-RESPONSE INCOMPATIBILITY TASKS. D. Ivkovich*, C. Christensen and K. Drake*. Department of Psychology, Vassar College, Poughkeepsie, NY 12601.

Twelve subjects were tested in an ERP paradigm to assess the effects of stimulus-response (S-R) incompatibility on reaction times (RTs) and P3 latencies. An earlier investigation (Pfefferbaum et. al., EEG clin. Neurophysiol., 64:424-437, 1986) had demonstrated that P3 latency is prolonged by S-R incompatibility, but only when the task predisposed to rapid responding. A more complex task using the same stimului but with a more complicated response rule showed no incompatibility effect on P3 latency. Slower, more deliberate responding characterized this task. To evaluate whether the P3-incompatibility effect can be manipulated by altering response strategy, subjects were tested on a simple and a complex S-R incompatibility task under three instructions emphasizing speed, accuracy, or a speed-accuracy tradeoff. The simple task consisted of compatibile trials in which the subject pressed one of two buttons as indicated by the words *RIGHT* or *LEFT* printed in upper or lower case; incompatible trials required pressing the opposite button. In the complex task, compatible responses were made to upper-case words while presentation of lower-case words required incompatible responses. As expected, RTs were influenced by task, incompatibility and instruction set, with prolongation for the more complex task, for incompatible trials, and increasing emphasis on accuracy. P3 latencies were also task dependent and influenced by instruction. Incompatibility prolonged P3 latency in the simple task. A lesser effect of incompatibility was observed in the complex task. The implications of these findings and the interaction of incompatibility and instruction in the two tasks are discussed in light of the hypothesis that the P3 S-R incompatibility effect is strategy dependent.

405.12

SHIFTS OF ATTENTION AND EVENT-RELATED POTENTIALS (ERPs) IN HUMANS: EFFECTS OF VALID AND INVALID CUEING. L. Anllo-Vento and M.R. Harter. Psychol. Dept. UNC-Greensboro, NC 27412.

A cue that indicates the most probable location of a subsequent target (valid cue) results in covert shifts of attention to that point in space. Shifts of attention, in turn, result in faster and more accurate detection at the target location. Single unit studies with monkeys and behavioral studies with human patients indicate that posterior parietal cortex is selectively involved in the directing of spatial attention. This study provides electrophysiological and behavioral measures of attention shifting in humans.

ERPs were recorded over occipital (O1-O2), parietal (P3-P4), central (C3-C4), and frontal (F3-F4) sites in response to validly and unvalidly cued targets appearing 14 degrees to the right or left of a central fixation point. The interval between the cue and the target varied randomly from 200 to 600 msec. The resulting waveforms indicate that attention can be switched within 350 msec. The amplitude of N1 to the target was modulated by the validity of the preceding cue, particularly at parietal and central sites. Amplitude of the P3 component reflected differences in probability of the valid and invalid conditions. Reaction times also revealed the costs and benefits of shifting attention to either the invalid or valid hemifield.

These results corroborate previous findings of parietal involvement in the directing of spatial attention.

405.13

HUMAN EVENT-RELATED POTENTIALS DURING SPATIAL PROCESSING: A TOPOGRAPHICAL DISTRIBUTION. G.F. Wilson, R.A. Swain* and I. Davis*. AAMRL, Wright-Patterson Air Force Base, Dayton, Oh. and SRL Inc., Dayton, Oh.

In the present experiment, we assessed the distribution and electrical characteristics of ERPs elicited during a variable demand spatial rotation task in which subjects were asked to decide whether a spatially rotated histogram was identical to an earlier template. Task difficulty was increased by incrementing both the number of bars in the histogram and the degree of rotation. Nine subjects were fitted with nylon caps containing 21 tin electrodes positioned in accord with the international 10-20 system. ERPs were recorded on a topographical mapping system (Bio-logic) and analyzed using the source derivation method developed by Hjorth (1975).

The results indicated that sensory evoked responses were evident at both central and temporal sites and that these potentials were insensitive to changes in task difficulty. Three parietal sites were associated with activity corresponding to the P200. As task difficulty increased, the activity recorded over the saggital suture showed decrements in both amplitude and latency. P300 activity was maximal at 3 parietal and 1 central (right hemisphere) sites. At all four sites, the P300 declined in amplitude as the task demand increased. Analysis of latencies indicated that the parietal peak latencies declined and that the right hemisphere central peak latency increased concurrent with cognitive demand.

405.14

INTRACRANIAL DISTRIBUTION OF HUMAN COGNITIVE POTENTIALS. M.E. Smith, E. Halgren, M. Sokolik,* P. Baudena,* A. Mussolino,* C. Liegeois-Chauvel,* and P. Chauvel.* INSERM U97, Centre Paul Broca, 75014 Paris, and VA Southwest Regional Epilepsy Center, Los Angeles.

Neocortical, limbic, and diencephalic regions have all been proposed as contributors to the P3 component of the ERP. In this study, the N2 and P3 potentials are recorded from epileptic patients undergoing SEEG. Recordings are made from parietal (n≥9 electrodes), temporal (n≥25), and frontal (n≥5) lobes. Electrodes have 5-15 contacts each, with over 700 contacts sampled. ERPs are recorded in an auditory oddball task, where the less frequently occurring of two distinct tones are counted mentally, and sometimes in a visual analogue to this task. Three main results have been obtained. The very large amplitude polarity inversions of the P3 that have been observed in the medial temporal lobe in prior studies are highly localized to the hippocampus itself, and seldom observed at more medial or lateral contacts. At neocortical sites, widespread normal polarity P3s and some small polarity-inverting P3s are observed. It is unclear to what degree these neocortical potentials are generated locally vs. volume-conducted. In contrast to the modality independent P3, recordings from superior-temporal and temporo-parietal areas reveal an N2 specific to the auditory modality.

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405.15

NEUROPSYCHOLOGICAL AND ELECTROPHYSIOLOGICAL DIFFERENCES IN TURNER SYNDROME. R. Clopper*[3], M. Schachter*[1], J. Shucard*[1], D. Shucard[1,2], Depts. of Neurology[1], Pediatrics[2], and Psychiatry[3], SUNY at Buffalo School of Medicine, Buffalo NY 14203.

In order to further clarify the cognitive phenotype in Turner Syndrome (TS), electrophysiological and behavioral measures were obtained in TS girls between the ages of 8 and 14 yrs. Subjects were evaluated with subtests of the Halstead-Reitan Battery to quantify spatial and sequential processing skills. Electrophysiological measures were obtained using auditory cortical evoked potentials (EP) to probe differences in electrical activity between homologous areas of the brain during the performance of visual-spatial tasks.

Neuropsychological findings indicated that although there was a heterogeneous pattern of test scores among subjects, they uniformly showed poor performance on the localization component of the Tactual Performance Test. Electrophysiological findings showed that the patterns EP asymmetry obtained during visual task performance appeared to be related to neurocognitive measures. These data indicate that neurocognitive deficits are present in TS but may be more complex than a general disturbance in spatial processing. Further, neurocognitive deficits may be related to electrophysiological indices of brain organization. Supported in part by Genentech, Inc.

405.16

FREQUENCY DEPENDENCY OF MIDDLE LATENCY AUDITORY RESPONSES OF HUMANS. M. L. Collaer *, R. A. Johnson and J. Beatty. Department of Psychology, UCLA, Los Angeles, CA 90024.

The P1 component of middle latency auditory event related responses is modulated by state of arousal and by stimulus repetition rate in both humans and cats. The neural generator of P1 is a matter of controversy, having been hypothesized to lie either within the rostral reticular activating system or in the primary auditory cortex.

P1 is a difficult component to analyze since its amplitude is relatively small and is often contaminated by muscle artifact. For these reasons, not all subjects demonstrate reliable P1 components.

We attempted to determine whether the neural tissue generating the human auditory P1 (occurring at latencies of 55-80 msec) is frequency dependent. Frequency dependency would be suggestive of a cortical rather than reticular source. P1s were measured to 800 Hz tone bursts, 100 msec in duration, presented at either 2 sec or 0.5 sec interstimulus intervals [ISIs]. It has previously been reported that P1 amplitude is attenuated at shorter ISIs. In the critical third condition, 800 Hz tone bursts were presented at 2 sec ISIs, with three 1000 Hz tone bursts intervening. Thus, in this condition, the frequency dependent ISI was 2.0 sec, while the frequency independant ISI was 0.5 sec.

Recordings were made on 11 subjects (bandpass filter settings of 10-300 Hz, Cz to linked ear reference, averages of 250 trials). P1 was measured relative to Nb, the preceding negativity. Identifiable and consistent P1s were detected in 5 of these individuals. The results suggest that the neural generators of the human P1 are frequency dependent: the amplitude of the P1 elicited by the 800 Hz tone in the mixed sequence was similar to that obtained by the 800 Hz presented at 2 sec ISIs. These results are suggestive of a cortical generator for P1. Investigations with additional subjects are being conducted. (Research supported by NIMH 37430-07)

405.17

STIMULUS INTENSITY EFFECTS ON MONKEY EVENT-RELATED POTENTIALS (ERPs): PARALLELS TO HUMAN AUGMENTING-REDUCING. T. C. Holmes[1*], J. A. Pineda[1], D. Swick[2*], and S. L. Foote[1,3]. (SPON: L. Adams). Depts. of Psychiatry[1] and Neuroscience[2], UCSD, and Scripps Clinic and Research Foundation[3], La Jolla, CA 92093.

Stimulus intensity effects on ERPs, as characterized by augmenting-reducing (AR), have been proposed to index a central mechanism for gating sensory processing and have been correlated with levels of biogenic amines. In order to study these effects, AR characteristics in auditory ERPs were recorded in squirrel monkeys (Saimiri sciureus) from chronically implanted epidural electrodes. The relationship between stimulus intensity and ERP component amplitude was examined at frontal midline, vertex, and temporal sites. Augmenting was defined as a monotonic increase in peak-to-peak amplitude for the first two major peaks, across three of the four intensities presented (50, 60, 70, and 80 dB SPL). Additionally, the response to the highest intensity was required to have the greatest amplitude. Any other result was defined as reducing. As in humans, differences in the AR profile were seen between electrodes. Frontal sites most commonly showed an augmenting profile, while temporal sites often showed a reducing profile. Despite amplitude variations between sessions, AR profiles were found to be stable over months for individual subjects. A Spearman rank-order correlation coefficient based on all subjects' data indicated a statistically significant correlation between amplitude/intensity slopes at temporal sites and P300 component areas elicited at parietal sites using an auditory oddball paradigm ($r_s \geq 0.90$, $p < 0.05$). These results indicate parallel findings in human and monkey ERPs with regard to AR profile differences between sites and P300-AR relationships.

405.18

EFFECTS OF CLONIDINE ON P300-LIKE POTENTIALS IN SQUIRREL MONKEYS. D. Swick[1*], J. A. Pineda[2], T. C. Holmes[2*], and S. L. Foote[2,3]. Depts. of Neuroscience[1] and Psychiatry[2], UCSD, La Jolla, CA 92093 and Scripps Clinic and Research Foundation[3], La Jolla, CA 92037.

Previous experiments in this lab have shown that bilateral lesions of the noradrenergic nucleus locus coeruleus (LC) decrease the amplitude of the monkey P300. To further assess the role of LC in modulating the orienting and attentional processes indexed by the P300, we systemically administered the alpha-2-adrenergic agonist clonidine to five squirrel monkeys (Saimiri sciureus) and recorded their event-related potentials (ERPs) from chronically implanted epidural electrodes. Two animals received extensive training in a 90-10 auditory oddball paradigm. Their P300 areas remained relatively stable throughout six pre-drug sessions and showed no habituation. Three untrained monkeys were presented with tones in a passive 80-10-10 oddball paradigm. In separate sessions, all animals received injections of saline or of clonidine (0.05, 0.075, and 0.1 mg/kg I.M., thought to suppress LC firing) and their ERPs were recorded. At lateral parietal sites, where the monkey P300 is most pronounced, three of five subjects (2 active, 1 passive) showed a dose-related decrease in P300 area and exhibited recovery to control levels in post-drug sessions. The remaining passive subjects failed to show clear P300s in both pre- and post-drug sessions, and hence their clonidine data were difficult to interpret. These preliminary results support the hypothesis that the LC noradrenergic system participates in P300 production.

405.19

IN SEARCH OF THE "ODDBALL": COGNITIVE PROCESSING OF STIMULUS SEQUENCE IN THE RAT HIPPOCAMPUS R.E.Hampson, C.R.Breese*, and S.A.Deadwyler, Dept. of Phys. & Pharm., Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC 27103.

In a continuously presented stimulus series in which the subject is instructed to detect one of two stimuli, the amplitude of the P_{300} component of the human scalp-evoked potential reflects expectancy of the stimulus as predicted by the immediately preceding sequence. The hippocampal formation has been suggested as the site of generation of the sequential dependency of P_{300}. A component of the tone-elicited hippocampal averaged evoked potential (AEP) in the rat has been identified that shows a sequential dependency in a manner similar to P_{300}. The N_1 component of the AEP can be described as an updatable buffer which stores the sequence of five preceding stimuli, but not the current stimulus (Deadwyler *et al.*, Behav. Neural Biol., 44, 1985). N_1 amplitude is maximal when preceded by a series of unrewarded (target) stimuli, and minimal when preceded by rewarded (nontarget) trials, irrespective of the current (evoking) stimulus. In contrast, P_{300} amplitude is maximal when the current stimulus is different from the preceding sequence of stimuli and does not depend upon whether the stimulus is a target or nontarget.

Recent studies from this laboratory suggest that P_{300} can not be generated in the hippocampus, but that the P_{300} process interacts with information represented by hippocampal sensory-evoked potentials (Hampson and Deadwyler, Behav. Brain Sci., 1988). This relationship provides the basis for a hypothesis that N_1 serves as a critical neurophysiological "substrate" for cognitive functions represented by P_{300}. The proposed interaction between N_1 and P_{300} will be examined using a neural modeling approach. A neural network model of serial memory reflected in N_1 amplitude and its extension to P_{300} will be discussed.
[Supported by NIDA grants DA04441, DA03502, and DA00119 to S.A.D.]

406.1

NEURAL FATTY ACID PROFILE IN WEANED RATS IS SUSCEPTIBLE TO SHORT-TERM CHANGES IN DIETARY FAT COMPOSITION. J.R. Dyer* and C.E. Greenwood (SPON: G.H. Anderson). Dept. Nutr. Sci., Fac. Med., Univ. Toronto, Toronto, Ont., Canada. M5S 1A8.

We have shown that dietary fatty acid (FA) composition, in the absence of essential fatty acid (EFA) deficiency, alters behaviour of weaned rats, including spatial memory, pain sensitivity and protein selection, within 4 weeks of feeding; i.e. neither long-term feeding for several months nor EFA deficiency were necessary to observe a functional effect of dietary fat. To determine if these behavioural changes were accompanied by altered lipid composition of brain membranes, the same 20% (w/w) soybean oil and lard diets were fed to 6 week old male Sprague Dawley rats for 4 weeks. Dietary fat influenced FA profiles of phospholipids (PL) in myelin, synaptosomes, mitochondria and microsomes; the degree of change was similar across all subcellular fractions and all PLs (PC, PS, PE, CL) except PI which was relatively unaffected. Polyunsaturated FAs, except arachidonic acid (C20:4ω6), were influenced by dietary fat more than saturated or monounsaturated FAs. The magnitude of change ranged from 5% for oleic (C18:1ω9) and 10% for docosahexaenoic (C22:6ω3) acids to 100% for linoleic (C18:2ω6) and 250% for docosapentaenoic (C22:5ω6) acids. The 22-carbon FA, C22:6ω3, characteristic of electrically excitable membranes, is believed to play a specific functional role. We hypothesized that dietary fat affects behaviour via altered neural membrane FA composition and subsequent functional changes in the membrane. (MRC).

406.2

CYTIDINE STIMULATES THE SYNTHESIS OF PHOSPHATIDYLCHOLINE IN A RAT PHEOCHROMOCYTOMA CELL LINE. I. Lopez G.-Coviella*, R. J. Wurtman, L. Robinson* (SPON: W. J. H. Nauta). Laboratory of Neuroendocrine Regulation, M. I. T., Cambridge, MA 02139.

Phosphatidylcholine, the most abundant phospholipid in cell membranes, is synthesized primarily via the CDP-choline pathway in most cell types. This route utilizes CTP and phosphocholine as its rate-limiting substrates. Thus, external factors that affect the availability of these precursors could influence phosphatidylcholine synthesis. We are investigating the effects of cytidine on cellular nucleotide content and on the incorporation of [^{14}C]-choline into phospholipids and water-soluble precursors. Rat pheochromocytoma cells (PC-12) were incubated in serum-containing D-MEM medium in the presence of [^{14}C]-choline (34 μM; 7.4 mCi/mmole), with and without cytidine (100 μM). After 15 and 30 minutes of incubation, incorporation of [^{14}C]-choline into phosphatidylcholine was increased by 55 and 42%, respectively, in cytidine-supplemented cells, while labeled phosphocholine levels were decreased (by 43% and 32%). Intracellular CTP levels were doubled after 15 minutes of incubation in the presence of cytidine, and rose even higher thereafter. In similar experiments, when cells were incubated with various concentrations of cytidine, these effects were dose and time-dependent. A pulse-chase study with labeled choline also showed that most of the radioactivity initially associated with phosphocholine was gradually converted to labeled phosphatidylcholine. In the presence of cytidine, this conversion was accelerated by 36 ± 4% at all times studied. These results suggest that cytidine could be a critical regulator of phosphatidylcholine biosynthesis; possibly by elevating intracellular levels of CTP.

406.3

MICROVESSEL MEMBRANE LIPID COMPOSITION AND AGING, W. M. Williams and T. H. McNeill, Dept. Neurology, Univ. of Rochester Sch. Med. Dent., Rochester, NY 14642.

Microvessels were isolated from the cerebral cortex and cerebellum of 10, 20 and 27-30 month old mice. Membrane lipids were extracted from both microvessel and brain parenchymal fractions, and phospholipids and cholesterol separated by thin-layer chromatography (TLC). The ethanolamine phosphoglyceride (EPG) spot was subjected to separation-reaction-separation TLC in order to separate the diacyl and plasmalogen (plasmene) fractions for analysis of fatty acids and dimethyl acetals. Methyl esters, and the aldehyde dimethyl acetals from the plasmalogen fraction were prepared and analyzed by gas-liquid chromatography (GLC). Total cholesterol was determined spectrophotometrically. The data suggests that, 1) microvessel membrane from 27-30 month old brain exhibits a marked increase in the level of unsaturation, attributable primarily to an apparent increase in arachidonic (20:4) and docosahexaenoic (22:6) acids, 2) cholesterol content increases in both microvessel and brain parenchymal membranes, and 3) in contrast to parenchyma, the relative content of the major phospholipid classes in microvessel membrane remains essentially unchanged over the age period studied. These findings suggest that microvessel endothelial membranes undergo acyl compositional changes that could potentially influence microvascular production of eicosanoids, and alter blood-brain barrier function.

406.4

ISOLATION OF THE JONES IMMUNOREACTIVE GANGLIOSIDE 9-O-ACETYL-GD3. D.M. Bonafede, L. Mecala*, M. Constentine-Paton and R.K. Yu. Dept. of Biology, Yale Univ. and Dept. of Neurology, Yale Med Sch., New Haven, CT 06511.

One of the 2 major gangliosides recognized by the monoclonal antibody JONES (Constantine-Paton et al. 1986, Nature 324: 459) is 9-O-acetyl-GD3 (Blum et al. 1987, PNAS 84: 8716). JONES immunoreactive gangliosides have been implicated in neural cell and process migration (Mendez-Otero et al. 1988, J. Neurosci. 8: 540) and intercellular interactions in vitro can modulate their expression (Mendez-Otero & Constantine-Paton; Bonafede & Constantine-Paton, 1987, Soc. Neurosci. Abstr. 455.6 &7).

We have used bovine buttermilk to isolate 9-O-acetyl-GD3 for functional studies. Our purification scheme was simplified with respect to standard procedures to minimize the number of steps required to obtain pure product. The procedure, after the initial extraction in chloroform / methanol, consists of 3 successive column chromatographies: first an ion-exchange column, then two silica gel (Iatrobeads) columns, all eluted with gradients of chloroform/methanol/ water. 9-O-acetyl-GD3 was identified on the basis of: 1) HPTLC motility between GM1 and GM2; 2) JONES immunoreactivity; 3) conversion to GD3 by mild base treatment.

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406.5

BIOCHEMICAL CHARACTERIZATION OF JONES IMMUNOREACTIVE GANGLIOSIDES IN RAT. P. Mylonas*, D.M. Bonafede, R.K. Yu and M. Constantine-Paton (SPON: J. Paton) Dept. of Biology, Yale Univ. and Dept. of Neurology, Yale Med. Sch., New Haven, CT 06511.

The monoclonal antibody JONES (Constantine-Paton et al. 1986, Nature 324: 459) recognizes a small family of rare gangliosides (Schlosshauer et al. 1988, J. Neurosci. 8: 550) in the developing rat nervous system. One of the 2 major gangliosides recognized is 9-O-acetyl-GD3 (Blum et al. 1987, PNAS 84: 8716).

We obtained, by ion-exchange chromatography, a ganglioside fraction enriched for the second major JONES immunoreactive ganglioside, which has HPTLC mobility between GD3 and GD1a. Mild base treatment of this fraction abolished JONES immunoreactivity and changed the HPTLC mobility of this ganglioside to the area just below GT1b, where tetrasialogangliosides run. The appearance of this band and the disappearance of the JONES reactive band near GD3, were the only changes produced by base treatment. This new band is recognized by the mAB A2B5, which was previously shown to be specific for GQ1c (Kasai & Yu 1983, Brain Res.: 277:155).

We conclude that the second (the first one being 9-O-acetyl-GD3) major JONES immunoreactive ganglioside is a base-labile GQ1c derivative, probably 9-O-acetyl-GQ1c.

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406.6

GANGLIOSIDE COMPOSITION OF CHOROID PLEXUS BRAIN TUMORS IN TRANSGENIC MICE. M. ElAbbadi, A. Messing and T. N. Seyfried. Dept. of Biol, Boston College, Chestnut Hill, MA 02167 and Sch. Vet. Med, Univ, WI.

The ganglioside composition of two choroid plexus tumors (papillomas), SV(188) and SV-MK(79) was examined in the brains of transgenic mice. These papillomas are inheritable tumors that develop spontaneously in adult transgenic mice carrying integrated copies of SV40 early region genes. The ganglioside composition of these tumors was compared to that of normal mouse brain and to a chemically induced ependymoblastoma growing subcutaneously in the flank. The total ganglioside sialic acid content (μg/100 mg dry weight) of the two papillomas was 76.2μg (four SV(88) tumors pooled for a single sample) and 139.4μg (the mean of two independent SV-MK(79) tumors). These concentrations were higher than that of the ependymoblastoma 39.4±3.1 (N=6), but markedly lower than the concentration in adult mouse brain (450-500μg). Although N-acetylneuraminic acid (NANA) is the only ganglioside sialic acid in adult mouse brain, both papillomas and the ependymoblastoma contained significant amounts of N-glycolylneuraminic acid (NGNA). GM3-NANA and GM3-NGNA were the predominant ganglioside species in both papillomas and the ependymoblastoma. From thin-layer chromatography evidence, the ceramide composition of the GM3 gangliosides appears to be more structurally homogeneous in the papillomas than in the ependymoblastoma. In contrast to the ependymoblastoma, the papillomas contained significant amounts of mouse brain gangliosides (GD1a, GD1b, GT1b, and GQ1b). These gangliosides may not be native to the tumors, but represent contaminants from normal brain tissue surrounding the tumors. (Supported by NIH grants NS-23355, NS-24826, and NS-22475).

406.7

GANGLIOSIDE MODULATION OF cAMP DEPENDENT PROTEIN KINASE AND CYCLIC NUCLEOTIDE PHOSPHODIESTERASE. A.J. Yates, J.D. Walters*, C.L. Wood*, S.M. Stock* and J.D. Johnson*. Departments of Pathology, Pharmacology, and Physiological Chemistry, College of Medicine and College of Dentistry, Ohio State University, Columbus, Ohio 43210.

Phosphorylation of proteins in membranes isolated from sciatic nerve of normal adult rabbits was examined using an in vitro assay, PAGE and autoradiography. In the absence (2 mM EGTA) of Ca^{+2} there were 3 major phosphorylated proteins (21 kDa,31 kDa and 51 kDa). Catalytic subunit of cAMP dependent protein kinase (PKA) enhanced phosphorylation of 21,31,38,40 and 49 kDa proteins. GM1, GD1a, GT1b inhibited phosphorylation by PKA of 38,40 and 49 kDa proteins with half maximal inhibition at 13-32 uM. With type IIA histone as a phosphate acceptor GM1 competetively inhibited PKA with Ki=110 uM. Neutral glycolipids were much less effective and free sialic acids had no inhibitory effect. GM1 stimulated the activity of purified bovine brain calmodulin-dependent cyclic nucleotide phosphodiesterase, with activation being half maximal at 0.3 uM and maximal (32-fold) at 1 um, but had no effect on Km. These findings indicate that gangliosides may affect the activity of PKA through two mechanisms: (a) decreasing levels of cAMP by activating phosphodiesterase; (b) direct inhibition of the catalytic subunit. Supported by NIH grant NS10165, Department of Pathology and College of Medicine.

406.8

MODULATION OF GAMMA-GLUTAMYL TRANSPEPTIDASE IN GLIAL CELLS BY GLUCOSAMINE. L.E. DeBault and J.L. Flagg-Newton*. Univ. of Oklahoma Health Sci. Cntr., Oklahoma City, OK 73190.

The amino-sugar, glucosamine, is known to decrease the expression of membrane-bound glycoproteins such as tyrosinase. However, cell surface gamma-glutamyl transpeptidase (GGTP) activity was stimulated by 15mM glucosamine in cultured glial cells (C_6, rat astrocytoma) over a 48 hr period. The increase in enzyme activity was dependent upon the entry of glucosamine into the cell and not on the inhibition of glucose transport. GGTP in glucosamine-treated cells exhibited an increased sensitivity to a variety of acceptor-substrates especially cysteine, while at the same time, enzyme affinity for wheat germ agglutinin (WGA) was markedly decreased. The enzyme also exhibited an altered Vmax. Rat liver hepatocytes (2B-RL), used as a control cell, had a similar Vmax alteration but in addition had an altered Km. The data suggest that the increase in GGTP activity in glucosamine-treated cells result from the appearance on the cell surface of a functionally distinct iso-form of GGTP. To date, it can be recognized by its disproportionate response to amino acid acceptors and by its decreased affinity for WGA. (This work was supported in part by NIH Grant R01-NS 18775)

406.9

ALTERATION OF INTRACELLULAR CHLORIDE CONCENTRATION AND ITS EFFECT ON THE EXPRESSION OF AChR IN MUSCLE. R.D. Heathcote and W.J. Betz. Dept. of Physiology, Univ. of Colorado School of Medicine, Denver, CO. 80262.

The mechanism by which muscle cell activity regulates the expresssion of acetylcholine receptors (AChRs) is not known. Expression of extrajunctional (EJ) AChRs correlates well with the level of internal [Cl] under a variety of conditions. For example, in normal muscle both are relatively low, but after denervation EJ AChRs appear and, as Cl conductance falls, internal [Cl] rises owing to the continued action of an inwardly directed Cl pump. Perhaps Cl acts as a second messenger, directing the expression of EJ AChRs. We tested this hypothesis by altering internal [Cl] in rat lumbrical muscles maintained in an organ culture system. Experimentally, AChRs were measured with ^{125}I-α-bungarotoxin binding, Cl conductance with electrophysiological methods and internal [Cl] with Cl-sensitive microelectrodes. Denervated muscles were cultured in low [Cl] medium (all but 9 mM Cl replaced by isethionate) to reduce internal [Cl]. This treatment inhibited AChR expression. However, low [Cl] medium caused extensive fibrillation, and when this was blocked by addition of tetrodotoxin, AChR expression rose to control levels, or higher. In the converse experiment, the appearance of AChRs was not accelerated when normal muscles were cultured in 9AC, a drug which blocks Cl conductance and raises internal [Cl]. We also investigated the effects of electrical stimulation of denervated muscle, which can restore AChR and internal [Cl] to normal levels. Addition of 9AC (which prevented the fall of internal [Cl]) did not prevent the loss of AChRs in electrically stimulated muscles. Thus, the observed increases and decreases in AChR expression are effected by altering muscle cell activity.

406.10

DENSITY OF ^{3}H-STX BINDING SITES IN PREMYELINATED AXONAL MEMBRANE IN MAMMALIAN OPTIC NERVE S.G. Waxman, J.A. Black and J.M. Ritchie*[1]. Depts. of Neurology and Pharmacology[1], Yale University School of Medicine, New Haven, CT 06510, and V.A. Medical Center, West Haven, CT 06510.

As part of a study on membrane assembly in myelinated axons, we studied STX-binding in premyelinated optic nerves (ON) of neonatal rat and rabbit. Since virtually all their axons become myelinated in the adult, these nerves provide a model for studying precursors of myelinated axolemma.

ON from neonatal (0-2 d) rats and rabbits were excised, weighed, pooled and homogenized, and the protein content and specific ^{3}H-STX-binding curve determined. The maximal specific STX-binding for neonatal rat and rabbit ON was 14 and 38 fmol/mg wet wt, respectively. Surface density of axon membrane, derived by the line-intersection method, was 9.6 $\mu m^2/\mu m^3$ (rat) and 11.3 $\mu m^2/\mu m^3$ (rabbit). For each species, surface density of ON glial membrane was ~10% of axon membrane surface density.

These results indicate a mean density of 4-7 STX binding sites per μm^2 of axolemma in neonatal rat and rabbit ON on the basis of a uniform plasmalemmal distribution of channels. Thus, both rat and rabbit premyelinated axolemma seem to exhibit an axolemmal Na channel density considerably lower than do adult unmyelinated fibers (100-200 μm^2). Since previous studies have demonstrated that neonatal rat ON exhibits TTX-sensitive action potential electrogenesis, the present results suggest that very low channel densities are capable of supporting spike electrogenesis in premyelinated fibers. [Supported in part by the NIH, NMSS and VA].

406.11

EXPRESSION OF GP50 BY CULTURED CEREBELLAR GRANULE CELLS. T. Paladino*, S.M. Nicholson*. P.W. Beesley*. and J.W. Gurd (SPON: I.R. Brown). Dept. of Biochemistry, Univ. of Toronto, Scarborough Campus, West Hill, Ont., M1C 1A4 and Dept. of Biochemistry, Royal Holloway and Bedford New College, Egham, Surrey, U.K. TW20 OEX.

We have previously described the brain-specific glycoprotein, GP50 and shown that it is expressed by granule cells in primary tissue culture (Beesley et al,1987, Br. Res., 408, 65-78). In order to further characterize GP50 we have investigated its developmental expression and molecular properties in granule cell cultures. Immunocytochemical staining of 10 day old cultures, using the monoclonal antibody Mab GP50 was confined to the perikaryal cell surface of granule cells present in cell clusters. Neurites showed little or no reactivity with Mab GP50. Immunoblotting showed that the levels of GP50 increased throughout the time in culture. In early cultures (<2 DIV) staining was reduced and was largely restricted to cells already present in forming aggregates. GP50 was labelled following the iodination of surface proteins with lactoperoxidase and partitioned (>70%) into the detergent phase following extraction with Triton X-114. Sucrose density gradient centrifugation of Triton X-100 extracts showed that GP50 exists predominantly (80%) in a 3.6S form (calculated Mr: 40K). The results indicate that GP50 is a developmentally regulated, integral, cell surface membrane glycoprotein. (Supported by grants from NSERC and MRC to JWG).

406.12

ASTROCYTE INVASIVENESS AND NEURON-SUBSTRATE CONTACTS IN FETAL RAT CNS CULTURES. V.H. Gilad, G. Shanker and G.M. Gilad. Dept. of Neurobiology, Coriell Inst. for Med. Res., Camden, NJ, USA.

In mixed fetal CNS cultures, growing astroglia express a membrane-associated activity that disrupt neuron-substrate interactions leading to neuronal detachment. The present study demonstrates first, that this invasive activity of astroglia can be prevented by treatment with 1mM dibutyryl cyclic AMP (cAMP), an ***in vitro*** morphogen that induces stellate morphology in astroglia. Second, the sulfated glycosaminoglycan heparan sulfate (HS) was found to inhibit neuron detachment in a reversible manner without affecting the flat morphology of astroglia. The importance of HS in neuron-substrate interaction was further elucidated by examining the effects of heparitinase. This enzyme which specifically hydrolyzes HS, led to neuronal detachment in the presence or absence of astroglia, but only during the early stage (2-3 days) of neuronal growth. Established cultures were not affected by heparitinase. We conclude: 1) the expression of astroglial invasive activity can be altered and may be dependent upon the state of differentiation, and 2) HS is probably involved in the formation of neuron-substrate contacts.

406.13

REGULATION OF PHOSPHATIDYLCHOLINE SYNTHESIS BY PHOSPHATIDYLSERINE AND PHORBOL ESTERS IN INTACT HUMAN NEUROBLASTOMA CELLS. B.E. Slack*, J.K. Blusztajn* and R.J. Wurtman. Laboratory of Neuroendocrine Regulation, Massachusetts Institute of Technology, Cambridge MA 02139.

Cultured neuroblastoma (LA-N-2) cells incubated in phosphatidylserine (PS) showed increases in PS content of cell lysates, and of subcellular membrane fractions. Treatment with PS (10 to 130 μM) but not PC, caused a concentration-dependent stimulation of up to two-fold in the incorporation of [^{14}C]choline into phosphatidylcholine (PC). PC synthesis can also be stimulated in many cell types by phorbol esters and diacylglycerols, both of which activate the calcium and phospholipid-dependent enzyme, protein kinase C. Phorbol myristate acetate (PMA, 100 nM) and dioctanoylglycerol (DiC_8, 1mM) increased PC synthesis by 14% and 50%, respectively, in untreated LA-N-2 cells. In cells previously exposed to PS, PMA and DiC_8 stimulated [^{14}C]choline incorporation into PC by approximately 35% and 120%, respectively, compared to cells treated with PS alone. The effects of PMA and DiC_8 were additive; together these compounds increased [^{14}C]choline incorporation in PS-treated cells by 200% with respect to PS-treatment alone. Under these conditions, the total increase in PC synthesis with respect to untreated controls was over 4-fold. In preliminary experiments cholinephosphotransferase activity was increased in cells pretreated with PS (10 to 30 μM). It remains to be determined whether PS treatment will also stimulate the rate-limiting enzyme cytidyltransferase. The results suggest that PS may stimulate PC synthesis by potentiating the activation of protein kinase C by phorbol esters and diacylglycerols. Our findings also indicate that formation of PS within the membrane may constitute a physiologic stimulus to overall membrane biosynthesis.

406.14

EFFECT OF CHRONIC ADMINISTRATION OF DIGITOXIN ON RETINAL SYNTHESIS OF NA PUMP ISOFORMS. S.C. Specht, José Martin*, Enid Gaud* and Sonia Castro Hernández*, Dept. of Pharmacology, Univ. PR School Med. San Juan, PR 00936.

The rat posseses at least three molecular isoforms of the catalytic subunit of the Na,K-ATPase which differ in sensitivity to inhibition by cardiotonic steroids. In order to determine if synthesis of the isoforms can be independently regulated by chronic administration of the cardiotonic steroids CD rats were treated for two weeks with daily injections (1.57 ml/kg) of digitoxin suspension (10 mM) in 1% carboxymethyl cellulose (CMC). Controls received only CMC. At the end of the treatment period, retinal proteins were labeled in vivo by intravitreal injection of 35S-methionine. The rats were killed after 2 hours. Na,K-ATPase was prepared from the retinae, the subunits were separated by SDS-PAGE and the radioactivity in the protein bands containing the alpha and alpha(+) forms were analyzed by liquid scintillation counting. The ratio of radioactivity in alpha(+) to alpha increased 13-20% in the digitoxin-treated animals. Incorporation into a broad group of proteins $\leq$ 58 kD was unaffected. The results demonstrate that synthesis of the catalytic subunits can be differentially regulated and suggest that chronic administration of digitoxin in a clinical setting may alter the usual cellular composition of Na pump isoforms.

406.15

ALTERED MEMBRANE COMPOSITION AND FLUIDITY IN SEIZURE-RESISTANT (C57BL/6J), SEIZURE-SUSCEPTIBLE (DBA/2J) AND KINDLED (C57BL/6J)MICE. G.M. Furman*, C. Applegate and R.A. Schatz, Toxicology Prog., Northeastern Univ. & Dept. Neuroscience, Children's Hosp., Boston, MA 02115.

Mice of the DBA/2J(D2) strain have an inherited susceptiblity to audiogenic seizures. C57BL/6J(B6) mice, however, are generally resistant to audiogenically-evoked seizures. The biochemical parameter underlying the difference in seizure susceptibility between these two strains has yet to be elucidated. This study aims to characterize D2 vs B6 mice by comparing the phospholipid (PL) and cholesterol (CL) content as well as the microviscosity of neuronal membranes from various brain regions. Alterations in membrane composition are reflected in the microviscosity of the lipid bilayer; modification of neuronal membrane fluidity may thus modulate the ability of neurons to fire and, in turn, influence seizure susceptibility. The fluidity of neuronal membranes of brain regions from both strains of mice was studied using fluorescence polarization and the PL/CL molar ratio, a biochemical estimate of fluidity. The cortex, hippocampus, striatum and midbrain from B6 mice were less fluid than corresponding regions in D2 animals as assessed by both techniques. Kindling of B6 seizure-resistant mice increased membrane fluidity in these regions as compared to non-kindled animals of the same strain. Thus, kindling altered the viscosity of neuronal membranes from seizure-resistant B6 mice such that they appear similar to those of seizure prone D2 mice. Taken together, the data suggest that a more fluid membrane microenvironment, whether genetically or electrically induced, may predispose neuronal membranes in some areas of the brain to hyperexcitability. This decrease in neuronal membrane viscosity may also be related to generation of ectopic foci and propagation of excessive electrical discharge in brain.

406.16

DEVELOPMENT OF MONOCLONAL ANTIBODIES TO THE SYNAPTIC MEMBRANE Ca^{2+}-Mg^{2+} ATPASE. M.L. Michaelis, T.E. Kitos*, S. Schueler* and L. Reichel*, Center for Biomed. Res., Univ. of Kansas, Lawrence, KS 66046

The regulation of free intraneuronal Ca^{2+} concentrations in the 0.1 uM range partially depends on the extrusion of Ca^{2+} via a (Ca^{2+} + Mg^{2+})-ATPase located in the neuronal plasma membrane. The properties of this enzyme in brain synaptic plasma membranes and its Ca^{2+} transporting activity have been described in earlier reports from our lab (Michaelis et al., J. Biol. Chem. 1983, 258, 6101-6108 and 1987, 262, 4182-4189. We have now developed methods to solubilize the enzyme in a Lubrol PX/Glycerol solution with $CaCl_2$ and NaCl, and to preserve enzymatic activity through the purification procedure. Calmodulin affinity chromatography was used to obtain a fraction exhibiting a 30-fold enrichment in Ca^{2+}-stimulated ATPase activity relative to that in the synaptic membranes. A silver-stained protein band with an estimated M_r of 140 kDa was greatly enriched on SDS-PAGE. This fraction was used to inject Balb C mice for preparation of hybridoma cells by standard fusion techniques. We now have at least 5 cloned hybridoma lines producing antibodies which recognize the isolated (Ca^{2+} + Mg^{2+}) ATPase. Further immunological characterization of these monoclonal antibodies is in progress. (DHHS grants AG 04762, AA 04732, and RR 5606, and DAMD 17-86-G-6038.)

406.17

THREE ISOZYMES OF THE Na,K-ATPase HAVE DISTINCTLY DIFFERENT CELLULAR DISTRIBUTIONS IN RAT CNS. K.M. McGrail* and K.J. Sweadner. Neurosurgical Research, Massachusetts General Hospital, Boston, MA 02114

Multiple isozymes of the Na,K-ATPase are present in the nervous system. Three alpha subunits have been sequenced, but little is known of their functions and cellular distributions. We used specific monoclonal antibodies for indirect immunofluorescence localization in the rat CNS. Markedly different patterns of staining were seen.

Specific stain for alpha 1 was found in the apical surface of the choroid plexus, in cuboidal epithelium lining the central canal of the spinal cord, and in vertically oriented fibers in the cerebral cortex. Staining for alpha 2 was present in many gray matter structures, but nearly absent in white matter tracts. Specific staining for alpha 3 was detected in pyramidal cells of the cerebral cortex, Purkinje cells of the cerebellum, and in axons of the brainstem and spinal cord.

Many different neuroanatomic structures and cell types stained for more than one isozyme. Most notable were the pyramidal cells of the hippocampus and the anterior horn cells of the spinal cord which stained intensely for alpha 2 and alpha 3. Dissociated Muller cells from the retina had both alpha 1 and alpha 2, while corpus callosum stained for alpha 1 and alpha 3. Even when a structure or region stained with more than one antibody the pattern of staining was frequently dissimilar, suggesting complex differences in cellular localization and gene expression.

406.18

PURIFICATION OF NA,K-ATPASE FROM HUMAN BRAINSTEM. J.H. Peng* and J.C. Parker, Jr.* (SPON: S.C. Sung). Dept. of Pathology, Univ. of Missouri-Kansas City Sch. of Med., Truman Med. Ctr., Kansas City, MO 64108.

The properties, structure and function of Na,K-ATPase in animal tissues has been studied extensively, but was rarely studied in human tissues. Hence, this study was undertaken to purify Na,K-ATPase from human brainstem, which will be used for biochemical and immunochemical characterization. Na,K-ATPase was purified from axolemma prepared from autopsy human brainstem tissue according to the established procedures with some modifications. Axolemma fractions were prepared sequentially by flotation technique, osmotic shock and discontinuous sucrose gradient centrifugation. Na,K-ATPase was then purified from axolemma by selective extraction using 0.4 mg SDS per mg protein for 30 min at room temp. The treated sample was diluted with medium immediately to reduce SDS concentration and layered over discontinuous sucrose gradients consisting of 6 ml of 1.0 M, 6 ml of 0.8 M and 10 ml of 0.5 M sucrose in ultra clear tubes and centrifuged at 27,000 rpm (Beckman SW 28 rotor) for 2 hr. Purified enzyme was settled at 0.8/0.5 M interface, which was collected by aspiration. The purified enzyme from human brainstem contained α+ and α subunits, corresponding to mol. wt. of 96,000 and 92,000 d, respectively, in contrast to that from rat axolemma, which has only α+ form. The purity of our enzyme preparation is more than 95% as determined by SDS-PAGE.

407.1

EXPRESSION OF TYROSINE HYDROXYLASE(TH)-IR AND mRNA IN RAT CHOLINERGIC CILIARY GANGLION NEURONS. S. Landis, A. Acheson and R. E. Siegel. Center for Neurosciences, Case Western Reserve Univ., Cleveland, OH. 44106.

The ciliary ganglion (CG) is classified as parasympathetic based on several criteria including location, acetylcholine synthesis and cholinergic target effects. Some CG neurons, however, are immunoreactive for TH, the rate-limiting enzyme in catecholamine synthesis (J Nsci 7:3574).

To learn more about TH expression and regulation in the CG, immunoblot and in situ hybridization studies were preformed. Comparison of immunoblots of CG and sympathetic ganglia revealed a 62kd band in both tissues. Analysis of IR/neuron suggested that similar amounts of TH were present in CG and sympathetic cell bodies. A higher molecular weight band was also detected in CG. In situ hybridization of CG sections with ^{35}S-labeled oligonucleotide probes showed that approximately the same proportion of CG neurons that exhibited TH-IR possessed TH mRNA. However, the TH mRNA levels were significantly lower in CG neurons than in sympathetic. The difference in the ratio of TH protein: mRNA in the two populations may reflect the absence of TH-IR in CG axons in normal adult rats. Chronic sympathectomy increases TH-IR in CG, results in detectable axonal transport of TH-IR and causes a significant increase in both the number of CG cells that contain TH mRNA and the amount per cell. By analogy with TH regulation in sympathetic neurons, NGF and impulse activity are likely candidates to mediate these effects. Supported by NINCDS 23678.

407.2

EXPRESSION OF SUBSTANCE-P (SP) BY SENSORY NEURONS OF DEVELOPING CHICK DORSAL ROOT GANGLION (DRG): ROLE OF CENTRAL AND PERIPHERAL CONNECTIONS. E: PHILIPPE*; C. DUC* and B. DROZ. Inst. Histol. Fac. of Med. Lausanne (Switzerland)

To determine the possible role of both the peripheral target tissues and the spinal cord on the expression of SP by sensory neurons, we studied DRG under the following experimental conditions: 1) When one hindlimb was resected in ovo at E 6 prior to the formation of specialized peripheral connections, the percentage of SP-immunoreactive ganglion cells (60 %), examined 6 days later, was not different from control DRG. However the intensity of immunostaining decreased.
2) When one hindlimb was resected at E 12 after establishment of peripheral connections, the immunostained cell bodies corresponded, 6 days later, to about 25 % of the neuronal population.
3) When the lumbosacral segment of the neural tube was destroyed by cauterization, the percentage of SP-immunoreactive cells examined 6 days later, was not different from the control DRG. These results suggest that SP-expression of sensory neurons may be modulated by peripheral target tissues but not by the spinal cord. (Swiss Nat.Science Foundation 3.447.83).

407.3

COMPARISON OF NEUROTRANSMITTER APPEARANCE IN THE PARACERVICAL GANGLION AND UTERINE CERVIX OF THE FEMALE RAT DURING DEVELOPMENT. K.A.Sullivan*, R.E.Papka and H.H. Traurig, Dept. of Anat. and Neurobiol., University of Kentucky, Lexington KY.

Our lab is currently interested in the development of the innervation to the female reproductive system. Acetylcholinesterase (ACHE) a marker for cholinergic neurons, tyrosine hydroxylase (TH) a marker for noradrenergic neurons, neuropeptide tyrosine (NPY) and vasoactive intestinal polypeptide (VIP) are present in the neurons of the adult paracervical ganglion (PG) and in axons in the adult uterine cervix. ACHE,TH,NPY,and VIP cell bodies are demonstrable in the PG on postnatal day 0. At this time ACHE,TH,NPY and VIP immunoreactivity is also seen in the cervix, mainly as bundles of preterminal axons. At early stages of development varicose fibers are not apparent but appear distinct by day 36. ACHE staining appears to increase in intensity in neurons and axons from days 0 to 36. There appears to be a relative decrease in the number of TH neurons and axons from day 0 to 36. The relative number of NPY and VIP neurons appears to increase from days 0 to 16. During this time NPY and VIP axons begin to branch, and form a mature appearing plexus by day 36.(Supported by NIH Grant NS-22526).

407.4

IMMUNOCYTOCHEMICAL LOCALIZATION OF PROTEIN KINASE C ISOZYMES IN RAT BRAIN. F. L. Huang*, Y. Yoshida*, H. Nakabayashi*, W. S. Young, III, and K.-P. Huang* (SPON: R. J. Waldbillig) NIH, Bethesda, MD 20892.

Recently we isolated three protein kinase C (PKC) isozymes from rat brain (PNAS 83 8535, 1986), and have determined the relative levels of each enzyme in various regions of rat brain (JBC 262, 15714, 1987). The present study describes the cellular distributions of PKC isozymes in rat brain as determined by light microscopic immunocytochemistry. Staining with PKC antibodies revealed strong immunoreactivities in neuronal somata and their dendrites. In cerebellum, the type I PKC antibodies stained the Purkinje cell bodies and dendrites; the type II PKC antibodies stained the granule cells; and the type III PKC antibody stained both Purkinje and granule cells. In the cerebral cortex, all antibodies stained neurons resembling pyramidal cells and their apical dendrites in layers II to VI, while layer I was nearly devoid of staining. However, staining with the various isozyme-specific antibodies revealed distinct laminar distribution patterns of the positively stained neurons. In the hippocampal formation, both pyramidal cells of the hippocampus and granule cells of the dentate gyrus were stained by all PKC antibodies. Subcellularly, type III PKC appeared mostly in the cytoplasm of these neurons whereas type I and II PKC seemed to associate with both cytoplasm and nucleus. The distinct cellular and subcellular distribution of PKC isozymes suggest that each isozyme plays an unique role in various neural functions. In developing cerebellum, cell type specific stainings indicated that the expression of PKC isozymes are differentially controlled and related to the various phases of cell proliferation and synaptogenesis during brain development.

407.5

EXPRESSION OF NPY, CGRP, AND NADPH-DIAPHORASE IN NEURONS OF THE DEVELOPING MURINE BOWEL: RELATIONSHIP TO THE APPEARANCE OF MUCOSAL 5-HT T.A.Branchek and M.D. Gershon, Dept. Anat. and Cell Biol. Columbia Univ. P&S, New York, N.Y. 10032.

The mechanisms underlying the generation of phenotypic diversity in enteric neurons are unknown. Serotonergic and cholinergic neurons appear earlier in ontogeny (day E12) than those which contain substance P or VIP (day E14), although substantial stores of endogenous 5-HT and neural 5-HT receptors are acquired long after serotonergic neurons can first be recognized. Enteric neural precursors continue to divide in the presence of mature neurons. These observations have led to the hypotheses that peptidergic neurons develop later than those that utilize small molecule transmitters and that early developing neurons affect neural development. In order to test these hypotheses we studied the phenotypic expression of neurons recognized by antisera to NPY and CGRP, and by the histochemical demonstration of NADPH-diaphorase. NPY immunoreactivity (IR) appeared for the first time on day E12, when it was seen in the stomach. NADPH-diaphorase, which is co-expressed with NPY-IR in many enteric neurons, was also found at this age. By day E13, the entire length of the bowel contained NPY-IR. In contrast, CGRP-IR could not be detected anywhere in the gut until day E17, but by day E18 all regions of the gut contained CGRP-IR neurons. Endogenous 5-HT was first detected at day E16 in mucosal epithelial cells in all segments of the bowel except the stomach, where it appeared at day E18. The NPY/NADPH diaphorase set of neurons thus develop before the acquisition of a detectable level of endogenous 5-HT or enteric neural 5-HT receptors. Consequently, small molecule transmitters do not necessarily develop earlier than peptides in enteric neurons and it is unlikely that 5-HT affects the differentiation of NPY/NADPH diaphorase neurons. Nevertheless, the possibility that 5-HT (or another early-appearing transmitter) might affect the development of CGRP-containing neurons has not been excluded. Supported by NIH grants # NS 22637, NS 15547, and NS 12969.

407.6

SPATIAL AND TEMPORAL EXPRESSION OF NEUROPEPTIDE mRNAs ENCODING MET-ENK, POMC, AND TRH: MAPPING IN XENOPUS LAEVIS USING *IN SITU* HYBRIDIZATION. W. P. Hayes, R. T. Zoeller and Y. P. Loh. LNN/NICHD, LNC/NINCDS, NIH, Bethesda, MD, 20892.

Neuropeptides have recently been implicated as trophic factors in neuronal development in addition to their well-documented neuromodulatory effects. To examine this possibility in early development, we have turned to the frog as a model system. *In situ* hybridization histochemistry was used to map neuropeptide mRNA expressing cells in adult and developing Xenopus laevis. Due to low amounts and rapid turnover of neuropeptides, *in situ* hybridization is a very sensitive cytochemical method for detecting neuropeptide expressing cells. 48-mer oligonucleotide, ^{35}S-labeled probes encoding regions of Xenopus-specific met-enkephalin (met-Enk), proopiomelanocortin (POMC), and thyrotropin releasing hormone (TRH) mRNA sequences were used to screen paraformaldehyde-fixed, cryoprotected, serially-sectioned frozen material.

Discrete patterns of hybridization for met-Enk, POMC and TRH mRNA were found in preoptic and hypothalamic areas in adult brain. In addition, POMC mRNA labeling was observed in cells confined within a tegmental nucleus, whereas cells labeled for met-Enk and TRH mRNA were differentially distributed in telencephalic areas, thalamus and motor nuclei. The distribution of labeled cells found in the CNS of Stage 45-46 tadpoles was similar to that found in adults. Earlier embryonic stages are being examined to determine when cells first express these neuropeptide mRNAs and whether cells express neuropeptides transiently. (W.P.H. and R.T.Z. are supported by Associateship grants from the National Research Council.)

407.7

THE SUBSTRATUM INFLUENCES NEUROTRANSMITTER EXPRESSION IN CULTURES FROM 3-DAY-OLD CHICK EMBRYO. D. Mangoura, N. Sakellaridis and A. Vernadakis.* Depts. of Psychiatry & Pharmacology, Univ. of Co. Sch. of Med. Denver, CO 80262.

We have reported: a) the developmental profiles of glutamate decarboxylase (GAD) and choline acetyltransferase (ChAT) bio- and immunocyto-chemically, for GABAergic and cholinergic neurons respectively, in neuroblast-enriched cultures from 3-day-old chick embryo, plated on poly-L-lysine in DMEM+10% FCS; and b) collagen as culture substratum inhibited neuronal aggregation and neuritic fasciculation. In this study, assessment of the same parameters, expressed as nmoles/mg protein/hr, for cultures on collagen revealed that while ChAT was not affected by the different substrata and thus the different cell organization, GAD was always markedly higher, increasing from culture day 3 (C3) up to C17 and remaining high up to C21. Similar effect was produced by 10ng NGF/ml of culture medium for neurons grown on poly. Immunostaining for GABA and ChAT reflected the biochemical findings. Overlapping of AChE and GABA staining indicated the presence of "cholinoceptive" GABAergic neurons on collagen. Glutamine synthetase and cyclic nucleotide phosphohydrolase, markers for astro- and oligodendro-cytes, showed very low, running in parallel profiles for both substrata, not related to GAD or ChAT peaks. These differences in GAD activity are attributed to factors secreted from nonneuronal cells, present in cultures on collagen, and/or cellular contacts shifting the GABA phenotypic expression (Support: MH15442).

407.8

DISSOCIATED CELL CULTURES OF GUINEA PIG CELIAC GANGLIA: AGE-DEPENDENT EXPRESSION OF NEUROPEPTIDE IMMUNOREACTIVITIES. D.L. Kreulen, R. Gruener and S.G. Matsumoto*. Depts. Pharmacology and Physiology, Univ. Ariz. Coll. Med., Tucson, AZ. 85724

We determined the relationship between expression of neuropeptides in culture and the age of the fetuses from which the neurons were derived. Neurons derived from the celiac ganglion of adult guinea pigs or fetuses at approximately 60% of term or greater exhibit immunoreactivities (IR) for neuropeptide Y (NPY) and somatostatin (SOM). Under standard culture conditions (ie. neurons grown alone) 60-80% of the neurons display NPY-IR and 10-30% SOM-IR when examined at 6 days to over 8 weeks in culture. Over 90% of the neurons also display tyrosine hydroxylase (TH)-IR and show glyoxylic acid-induced catecholamine histofluorescence (GIF).

Cultures established from earlier developmental stages showed a greatly reduced proportion of NPY-IR (20-40%) and SOM-IR (2-5%) neurons. The number of TH-IR and GIF positive neurons remains high (>90%). Many of these neurons make cholinergic synapses upon themselves and on other neurons when they are co-cultured with cardiac myocytes in microcultures.

Supported in part by NIH HL27781, HL01136 (DLK) and NIH 25644 (SGM).

407.9

POSTNATAL DEVELOPMENT OF TYROSINE HYDROXYLASE IMMUNOREACTIVE NEURONS IN THE MOUSE CNS. J.Satoh* and K.Suzuki. Dept. of Pathology, Sch. of Med., Univ. of North Carolina, Chapel Hill, NC 27599-7525

Using polyclonal antiserum to tyrosine hydroxylase (TH, Eugene Tech, NJ), we studied expression of TH-like immunoreactivity in CNS neurons during postnatal development in the mouse. Swiss Webster albino mice (from P2 to P40) were anesthetized and perfused with 2.5% glutaraldehyde / 2% paraformaldehyde. 50μm vibratome sections were treated with 1% sodium borohydride (Kosaka et al, Neurosci 18: 957, 1986) and immunostained with avidin-biotin complex method. TH-like immunoreactive neurons (TH-IN) were detected in substantia nigra, locus ceruleus, arcuate nucleus and pyriform cortex in all age groups. In neocortex, between P8 and P18, many intensely positive TH-IN were found in the medial (anterior and posterior), frontal and temporal regions of neocortex. Intensely stained neurons were pronounced in the cingulate and the cortex lateral to the striatum. Most TH-IN were multipolar and located in the deep cortical layers. Immunoreactivity of TH-IN decreased significantly after P18 and was hardly detectable at P40. Transient enhancement of immunoreactivity was also noted in TH-IN in the inferior colliculus. Similar transient expression of TH-IN during postnatal period has been reported (Jaeger et al, Dev Brain Res 1:128, 1983; Burger et al, Dev Brain Res 3:141, 1985) in the rat. Transient "overshoot" of TH activity between P13 and P18 was reported in the mouse (Baker et al, Dev Brain Res 4:157, 1982). These observations suggest the importance of the catecholamine system in this particular period of brain development. Our preliminary observation on increased numbers and intensity of TH-IN in mice treated with disulfiram during this period suggests that perturbations of the brain catecholamine system may be expressed as changes in TH-IN.

407.10

AMOUNTS OF TYROSINE HYDROXYLASE mRNA DURING MATURATION OF RAT HYPOTHALAMUS, SUBSTANTIA NIGRA, SUPERIOR CERVICAL GANGLION AND ADRENAL. W. Kedzierski* and J.C. Porter, Dept. OB/Gyn and Physiol., Univ. of TX Southwestern Medical Center, 5323 Harry Hines Blvd. Dallas, TX 75235

The purpose of this study is to investigate the effect of age on tyrosine hydroxylase (TH) mRNA in catecholamine-secreting cells. TH mRNA was analyzed by an S1 nuclease assay utilizing a [^{32}P]-labelled RNA probe (cRNA) that is complementary to TH mRNA. TH mRNA was extracted from tissue using guanidinium thiocyanate-phenol and hybridized with [^{32}P]cRNA. The product was subjected to S1 nuclease digestion. The TH mRNA-[^{32}P]cRNA duplex was dissociated, and the protected [^{32}P]cRNA was purified by urea PAGE. An autoradiogram of the gel was analyzed by densitometry. TH sense RNA, which served as a standard, was treated similarly. TH mRNA increases five to sixfold between 4 days and 6 weeks of age in the hypothalamus, substantia nigra, and adrenal (Table 1). Further development does not change the level of TH mRNA in the brain. However, between 6 weeks and 12 weeks, TH mRNA in the adrenal increased twofold.

Table 1. TH mRNA (amoles) in maturing male rats.

Age	Hypo-thalamus	Substantia nigra	Sup.cerv. ganglion	Adrenal
4 days	7.5 ± .4	107 ± 2	--	62 ± 3
6 wks	45 ± 10	480 ± 25	215 ± 14	378 ± 21
12 wks	51 ± 8	478 ± 13	274 ± 11	865 ± 30

407.11

ADRENERGIC DIFFERENTIATION IN QUAIL DORSAL ROOT GANGLION CELLS CAN BE INITIATED BY INSULIN AND IGF-I. J. Smith*, Z.G. Xue* and N.M. Le Douarin, Institut d'Embryologie Cellulaire et Moléculaire du CNRS et du Collège de France, 94736 Nogent-sur-Marne, France.

Avian dorsal root ganglia (DRG) contain a population of cells that are able, when cultured in vitro, to express a catecholaminergic phenotype, displaying tyrosine hydroxylase (TH) immunoreactivity and synthesising noradrenaline (Xue, Z.G. et al., Proc. Natl. Acad. Sci. USA 82:8800, 1985). In situ hybridisation with a quail TH-cDNA probe (Fauquet, M. et al., J. Neurochem. 50:142, 1988) reveals that TH mRNA is first expressed on day 3 of culture, at virtually the same time as the enzyme becomes detectable immunocytochemically. Catecholaminergic differentiation in this system is highly dependent on the presence of chick embryo extract (CEE). We have used quail DRG cultures, grown without CEE, to assay various hormones and growth factors for catecholaminergic differentiation-promoting activity. EGF, FGF, NGF, hydrocortisone and tri-iodothyronine are, at best, only slightly effective. In contrast, nanomolar concentrations of insulin or IGF-I elicit the development of as many TH-positive cells as do optimal concentrations of CEE. In all respects (phenotypic characteristics of the cells, their emergence from dividing precursors, the kinetics of appearance of TH mRNA and protein), the catecholaminergic differentiation observed in the presence of insulin appears identical to that occurring in medium containing CEE. We suggest that insulin-like polypeptides are plausible candidates for a role in triggering the normal development of sympathoblast precursors in vivo.

407.12

CHARACTERIZATION OF TYROSINE HYDROXYLASE (TH)-POSITIVE CELLS CULTURED *IN VITRO* FROM EMBRYONIC CHICK SPINAL CORD. J.A. Wallace, P.C. Allgood*, R.R. Maez*, A.A. Romero*, A.J. Valleios*, I. Lopez-Colberg and A.C. Towle. Dept. of Anatomy, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

Numerous TH-immunoreactive cells occur in the embryonic chick spinal cord, both ventral to the central canal and along the lateral border of the dorsal horn (Wallace et al., Neurosci. Letts., 83:253, '87). While only occasional cells are detected on embryonic day 15 (E15), these cells quickly reach their full complement in numbers by E17. When examined by GA histofluorescence, these TH-positive spinal cord cells are catecholamine (CA)-negative, although in other species they have been shown to contain dopamine (DA). In the present study, we examined the development of the TH-positive cells grown in vitro. Dissociated cells from chick spinal cords from E8 or E10 embryos were incubated for varying lengths of time on collagen-coated plastic slides. When cultured for 8 days, large numbers of TH-immunostained cells are present. Cells are also detected by anti-DA immunocytochemistry, although their number represents less than 10% of TH-stained neurons found in sister cultures. When the cultures are incubated for shorter periods (approximating embryonic in ovo ages earlier than E15), very few TH-containing cells are found. Thus, the relative timing of appearance of TH-positive cells grown in vitro closely mimics that observed in vivo. This model system may allow an examination of factors influencing CA neurotransmitter phenotype expression in the developing CNS. (Funded by NSF BNS-8511079 and NIH RR-08139.)

407.13

ONTOGENY OF THE PROTHORACICOTROPES IN THE TOBACCO HORNWORM, MANDUCA SEXTA. A.L. Westbrook* and W.E. Bollenbacher. Dept. of Biology, CB# 3280, Coker Hall, University of North Carolina, Chapel Hill, NC 27599-3280.

Prothoracicotropic hormone (PTTH) is a neuropeptide regulator of insect molting and metamorphosis. The expression of PTTH during embryogenesis in Manduca sexta was examined immunocytologically using a monoclonal antibody against PTTH. The neuropeptide was first expressed by the 4 lateral cerebral neurosecretory cells (L-NSC III) between 25-30% of embryonic development. The developing axons of these NSC traversed the cerebral lobes and decussated to the contralateral lobe (approximately 45% development), and exited the brain via the nervus corporis cardiacum I+II (approximately 60% development). Growth of dendritic processes within the protocerebral neuropil began at approximately 60% development, and their complexity increased up to hatching. By approximately 75% embryonic development, the axons had reached the corpora allata and arborized forming the terminal varicosities of the neurohemal organ. In addition to the L-NSC III, transient PTTH-like immunoreactivity was observed in the frontal and subesophageal ganglia, as well as the lateral brain regions.

407.14

EARLY DEVELOPMENT OF GABA IMMUNOREACTIVE NEURONS IN CEREBRAL CORTEX OF FETAL MONKEYS. M.L. Schwartz and P.S. Goldman-Rakic. Sect. of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510

Many features of cortical connections in monkeys are well specified by the latter third of gestation. Here we have examined the development of GABA-containing local circuit neurons in the macaque prefrontal cortex (PFC). Sections of dorsolateral PFC from 15 fetal monkeys were processed for GABA (Incstar) immunoreactivity. As early as embryonic day 55 (E55) of the 165 day gestational period, GABA immunoreactive neurons (GINs) are present within the cortical plate (CP), the underlying subplate (SP) and the intermediate zone (IZ). The majority of GINs form a band spanning lower layer VI and the upper portion of the SP/IZ. SP/IZ GINs are multipolar or have an elongated soma with leading and trailing processes, the latter resembling migrating cortical neurons. Between E55 and E72 the density of GINs increases and cells in the subplate and lower layer VI develop elaborate processes. GINs are now present throughout the CP; their density increasing from superficial to deep with the exception of the high density in the marginal zone. Between E72 and E116 their density in the IZ/SP diminishes. At E116 all GINs are non-pyramidal in morphology and are densest in layers I and VI. The E116 pattern is not fully mature in that relatively few labeled cells are found in layer II and upper layer III. Between E116 and E131 two dense bands of labeled cells emerge: one spanning layers I, II and upper layer III and the other in layer IV. GINs in the emerging white matter are now fewer in number and the overall laminar pattern is similar to that of mature monkeys. These results indicate that many neurons of the developing cortex express GABA within the first third of gestation. Further, their morphology and early appearance in the IZ and SP suggest that GABA may be expressed in some cells during migration. Supported by BNS8617585,MH38546 and NS22807

407.15

LAMINAR DISTRIBUTION OF $GABA_A$ RECEPTOR AND GABA IMMUNOREACTIVITIES DURING DEVELOPMENT OF RAT CEREBRAL CORTEX. A. Cobas*, G. Alvarez-Bolado*, A.L. DeBlas and A. Fairén* (SPON: European Neuroscience Association). Instituto Cajal, CSIC, 28006 Madrid, Spain, and Department of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Rats aged from E16 through P33 were processed for the immunocytochemical localization of GABA (Seguela et al., 1984, PNAS, 81:3888-92) and $GABA_A$ receptor (mAb 62-3G1: de Blas et al., 1988, J. Neurosci., 8:602-14; Vitorica et al., 1988, J. Neurosci., 8:615-22). At E16, $GABA^+$ cells are abundant at the lower intermediate zone and at both sides of the cortical plate (CP), which is also outlined by two narrow bands of $GABA_A$ immunoreactivity. With advancing development, the marginal zone soon becomes the most densely immunoreactive lamina for the $GABA_A$ receptor. In perinatal animals, the neuropil between the inferior border of the CP and the superior limit of the white matter shows a pattern of $GABA_A$ immunoreactivity characterized by laminar differences in intensity. $GABA^+$ cells are most abundant at this level. The laminae of $GABA_A$ immunoreactivity gradually become less distinct during the next stages of postnatal development. At P33, the densest $GABA_A$ immunostaining is in layer I, the rest of the layers showing a rather uniform pattern of staining.

With the exception of the earliest stages, a parallelism exists between the laminar patterns of $GABA_A$ and GABA immunoreactivities observed during cortical development.

407.16

ANGIOGENESIS IN THE OPTIC TECTUM OF XENOPUS TADPOLES. C.M. Rovainen. Dept. Cell Biol. and Physiol., Washington Univ. Sch. Med., St. Louis, MO 63110

The goal of this work has been to develop an amphibian model for the growth of capillaries of the CNS. Anesthetized tadpoles of albino Xenopus laevis are sufficiently transparent that individual capillaries, blood cells, endothelial nuclei, and sprouts can be resolved on the surface of the optic tectum in vivo by conventional light microscopy.

1. Case histories indicated the pial capillaries developed by the classical mechanism of sprouting of endothelial cells from existing blood vessels (Eliot Clark, 1918).
2. "Deep sources" apparently developed from surface capillary sprouts that penetrated the basement membrane, invaded the nervous tissue, and joined more arterial vessels in the ventral brain.
3. The dorsal medial venules enlarged in diameter as they drained an increasing blood flow from the tectum into the sinuses of the caudal choroid plexus.
4. Some capillaries disappeared during development. Early signs of regression appeared to be cessation of flow, narrowing, and loss of contrast. A common site of regression was upstream from a deep source.
5. No significant differences in densities of surface vessels or of deep sources were observed after removal of one eye, rearing at 30° or 12°, or rearing at half atmospheric pressure or in high O_2.

407.17

TYROSINE HYDROXYLASE IMMUNOREACTIVITY AND DOPAMINE SYNTHESIS PRECEDE DOPAMINE UPTAKE IN MESENCEPHALIC DOPAMINERGIC NEURONS FROM RAT EMBRYOS. M.L. Fiszman*, A. Zuddas, J.L. Barker and U. di Porzio. Laboratory of NeurophysIology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

In this report we show that in vivo the development of tyrosine hydroxylase (TH) immunoreactivity and dopamine (DA) synthesis in mesencephalic DA neurons from rat embryos takes place before the onset of DA uptake system. Ventral mesencephali from embryonic day 11 (E11) to 1 week after birth were dissected and either processed for determination of endogenous CA by HPLC or dissociated after papain digestion (20 units/ml, 45 min/37°C) and plated in polylysine coated dishes. Cells were allowed to adhere to the plate for five hours and either fixed (4% paraformaldehyde) for tyrosine hydroxylase immunostaining or incubated with 50nM of ^{3}H-DA for 30 min/37°C or 0°C (blanks) for uptake experiments. DA begins to be detectable by HPLC in the mesencephalic homogenates at E13-E14 (about 5% of E19 content) but could not be determined in the striatal tissue before E16-E17 (1-3 pg/mg protein). TH-immunoreactivity is already present in E12 cells (putative DA neurons) and the number of TH^+ neurons increases thereafter. Quantitative data on the development of TH^+ neurons will be presented. The DA uptake mechanism appears in these cells between E15 and E16 and its capacity increases with embryonic age. The maturation of DA uptake in DA mesencephalic neurons appears therefore coincidental with the beginning of DA innervation in the striatum thus suggesting that its development is regulated by the contact with target striatal neurons, as previously observed in culture (PNAS 76, 5387, 1979; Nature 288, 370, 1980).

407.18

GABA, NEUROPEPTIDE, AND TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE FRONTAL CORTEX OF FETAL MONKEYS. G.W. Huntley, S.H.C. Hendry, H.B. Killackey, L.M. Chalupa and E.G. Jones. Depts. of Anatomy and Neurobiology and Psychobiology, University of California, Irvine, CA. 92717 and Dept. of Psychology, University of California, Davis, CA. 95616.

The frontal lobes from six fetal rhesus monkeys (Macaca mulatta) were used to examine the morphology and distribution of immunocytochemically identified GABA and neuropeptide containing neurons and to study the distribution of tyrosine hydroxylase-immunoreactive (ir) fibers during the third trimester of gestation (E110,121,131,135,150,155). All ir-cell and fiber populations were present from the youngest age. In each of the fetuses, GABA-ir neurons were present in all layers and in the subcortical white matter, but were densest in a superficial band (layers I-II) and in a deep band (layer IV). Neurons ir for NPY and SRIF were scattered throughout layers II-VI, but were densest in the underlying white matter. SP-ir somata occupied a thin band corresponding to layer V. Proenkephalin A (BAM-18)-ir neurons were located in the upper half of layer II. TH-ir fibers were densest in layers I-III and in layer VI and the subjacent white matter. Additionally, occasional TH-ir somata were present in layer VI of the developing frontal cortex. The patterns of cell and fiber immunostaining are unlike those in the adult, and show significant changes in morphology, distribution and density over the gestational period examined. Supported by Grants EY 06432, NS 21377 and RR 00169 from the National Institutes of Health.

408.1

EFFECTS OF IONTOPHORETICALLY APPLIED U50,488H (A KAPPA OPIATE AGONIST) IN THE SUBSTANTIA NIGRA. L.A. Thompson and J.M. Walker. Department of Psychology, Brown University, Providence, RI 02912.

The presence of a prodynorphinergic striatonigral pathway and the presence of kappa opiate receptors in the substantia nigra (SN) suggest a physiological action of kappa-selective peptides in this area. In order to investigate specific kappa actions in the SN, the selective agonist, U50,488h, was applied by microiontophoresis during extracellular single unit recording of neurons in the SN pars compacta (SNC) and the SN pars reticulata (SNR) in rats.

U50,488h had no effect on dopaminergic neurons of the SNC and predominantly inhibited the spontaneous firing of neurons in the SNR. Approximately 50% of SNR neurons tested were inhibited by U50,488h, and this effect was dose dependent. U50,488h appears to differentially affect SNR neurons that are distinguished by their response to a mechanical pressure stimulus applied to the hindpaw. Cells that exhibit an increase in firing rate in response to mechanical pressure are inhibited by U50,488h more often than cells that exhibit a decrease in firing in response to the stimulus or that are unaffected. These results support previous findings from this laboratory that the systemic administration of U50,488h differentially inhibits SNR neurons that exhibit excitation in response to mechanical pressure.

408.2

RECEPTOR BINDING PATTERNS IN THE BASAL GANGLIA OF PIGEONS E.K. Richfield, R.L. Albin, A. Reiner, A.B. Young and J.B. Penney, Jr. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI and Dept. of Anatomy and Neurobiology, Univ. of Tennessee., Memphis, TN.

Recent studies have shown that the basal ganglia in birds consists of fundamentally the same neuronal populations as in mammals, with the neurons of each population being definable in terms of their connections and neurotransmitter content. These results have suggested a high degree of similarity in functional organization of the basal ganglia among birds and mammals. To further explore this similarity, we have used autoradiographic techniques to examine the pigeon basal ganglia for the presence and distribution of several different receptor types that bind neurotransmitters known to be present in avian basal ganglia and its projection systems.

The following receptor types were examined (with the radiolabeled ligand used for each in parentheses): 1) D-1 dopamine (SCH23390); 2) D-2 dopamine (spiperone); 3) muscarinic cholinergic (QNB); 4) NMDA (glutamate blocked with cold quisqualate); 5) quisqualate (QA) (glutamate blocked with cold NMDA); 6) GABA-A (GABA blocked with cold baclofen); 7) GABA-B (GABA blocked with cold isoguvacine); and 8) benzodiazepine (BDZ) (flunitrazepam). The striatum was found to be rich in D-1, D-2, muscarinic, NMDA, QA, GABA-A and BDZ receptors. Of the target areas of the striatum, the pallidum was rich in GABA-A and BDZ receptors, while the nigra contained moderate levels of D-1, D-2, muscarinic, GABA-A and BDZ receptors. These results are similar to those in mammals and are consistent with the view that neurotransmitters previously localized to the pigeon basal ganglia play roles very similar to the roles they play in mammals. Supported by NS-19620 (A.R.) and NS-19613 (A.B.Y.).

408.3

NEOSTRIATAL AND FRONTAL CORTICAL CATECHOLAMINE CELLS IN LONG-TAILED MACAQUES: INDIVIDUAL VARIATION AND LESION-INDUCED OVERDEVELOPMENT. Mark Dubach, Richard H. Schmidt, Douglas M. Bowden. Psychiatry and Behavioral Sciences, Neurological Surgery, and Regional Primate Research Center, University of Washington, Seattle WA 98195.

We have reported a population of neostriatal neurons containing tyrosine hydroxylase-like immunoreactivity (TH-LI) (Neurosci Lett 75:205). We have now found these cells to be positive for dopa decarboxylase, but not for dopamine beta-hydroxylase, suggesting dopamine as their neurotransmitter. Furthermore, we have detected a similar population of cells in the medial frontal cortex (subcallosal gyrus, gyrus rectus) distributed (as in the striatum) near the border between white and gray matter.

In some monkeys, however, we have seen fewer positive cells than in others. To examine such variability while minimizing technical variations in processing, we performed immunohistochemistry on tissues from six animals simultaneously, treating comparable sections from each of twelve hemispheres together in each container. Results indicate a wide range of variability among individuals. In several animals, including three of those treated simultaneously, we have examined these cells as they appear in striatum denervated of dopaminergic input by a lesion of the substantia nigra. Striatal TH-LI cells under these conditions appear to possess thicker and more extensively ramified processes than cells in normal striata. Analysis by densitometry and quantitative microscopy suggests that the overdevelopment is correlated with diminished nigral input. The time-course of this neuronal plasticity after 6-OHDA and electrolytic lesions is now under investigation. (USPHS grants NS25724 and RR00166).

408.4

ULTRASTRUCTURE OF THE MARGINAL DIVISION OF THE STRIATUM IN THE RAT. S.Y. Shu*, C.E. Bland, and T.M. Charles. East Carolina University, Greenville, NC 27858. U.S.A. *Department of Neurobiology, Fourth Military Medical College, Xian, China.

In our former study, a new subdivision of the striatum, the marginal division was found in the rat (Shu et al. J. of Chemical Neuroanatomy 1988). The marginal division is a narrow band located at the caudal margin of the striatum surrounding the rostral and lateral border of the globus pallidus. Neurochemically, the marginal division is lighter in acetylcholinesterase staining and more densely filled with Met-enkephalin-, substance P-, and dynorphin B- immunoreactive fibers and terminals than the other part of the striatum.

This study investigates the ultrastructure of the marginal division of the striatum in the rat. Morphologically, the neurons in the marginal division are oval with a medium amount of cytoplasm which is rich in rough endoplasmic reticulum and mitochondria. The nuclei of these neurons are also oval-shaped without indentation. There are many types of synapses in the marginal division. These are axo-dendritic (A —> D, A —> D <— A, D <— A —> D), axospinous and axo-somatic. Most of them are asymmetrical synapses. The multiplex of synapses indicates the functional complexity of the marginal division in the striatum.

408.5

DESCENDING PROJECTIONS FROM SUBSTANTIA NIGRA TO THE MEDULLARY RETICULAR FORMATION. M. von Krosigk* and A.D. Smith. MRC Anatomical Neuropharmacology Unit, University Dept. Pharmacology, South Parks Road, Oxford, OX1 3QT, UK.

Using both retrograde and anterograde neuroanatomical tracers we report a descending projection from the substantia nigra to the medullary reticular formation of the rat. The retrograde tracer Wheat Germ Agglutinin Horseradish Peroxidase (1-5%, Sigma) was injected (8-100 nl) into the medullary reticular formation. These injections revealed the presence of retrogradely labelled cells in both substantia nigra pars compacta and pars reticulata, SNc and SNr, respectively, with the majority of cells found in SNr. Within SNr the labelled cells were restricted to the caudal and lateral half of SNr. Injections of the anterograde tracer Phaseolus Vulgaris Leucoagglutinin into the caudal and lateral substantia nigra revealed a medullary projection which was restricted to the lateral parvocellular reticular formation, PCRt. This anterograde labelling ran throughout the rostrocaudal extent of the PCRt. Also labelled was the rostral region of the nucleus of the solitary tract, NTS. Caudally, the labelling in the NTS was restricted to the ventrolateral NTS, dorsally adjacent to the PCRt.

408.6

SUBTRACTIVE cDNA CLONING OF NEURONALLY EXPRESSED mRNAs ENRICHED IN RAT CAUDATE PUTAMEN. Joseph B. Watson* and J. Gregor Sutcliffe (SPON: S. Forss-Petter). Molecular Biology Department, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Precise knowledge of the neuronal organization of the neostriatum (caudate nucleus, putamen) will help to better define its role in voluntary movement and may suggest hypotheses to explain striatal pathologies (e.g. Huntington's Disease). One important task is to describe the presently known striatal cell types in more molecular detail. Toward this end, we are systematically isolating cDNA clones (such as pRC3) of mRNAs with enriched expression in rat caudate putamen. RC3 detects two postnatally expressed mRNAs (1.0 kb, 1.5 kb) prevalent in striatum, cerebral cortex, hippocampus, much less abundant in olfactory bulb, pons, and undetectable in cerebellum. Translation of a full length cDNA sequence predicts a novel 9.4 kDa protein with a cysteine-rich domain at its NH_2-terminus similar to cobra venom neurotoxins and C5a anaphylatoxin, and a glycine-rich domain at the COOH-terminus similar to that found in keratin. In situ hybridization experiments show that RC3 mRNA is expressed in restricted neuronal populations of the telencephalon and diencephalon. There is elevated expression in medium-size neurons of the caudate putamen over globus pallidus suggesting that RC3 may be a marker for a class of striatal neurons. Analysis of additional cDNA clones detected by subtractive hybridization using a rat caudate putamen-enriched cDNA probe will also be presented.

408.7

EFFECTS OF INTRASTRIATAL ESTROGENS ON THE DORSAL IMMOBILITY RESPONSE IN GONADECTOMIZED MALE AND FEMALE RATS. C.Van Hartesveldt, G.A. Cottrell and M.E. Meyer*. Psychology Dept., Univ. Florida, Gainesville, FL 32611.

Previous research has shown that in OVX females, intrastriatal estradiol significantly potentiates the dorsal immobility response (DIR), a response elicited by lifting the rat off its feet by the skin at the nape of its neck. We investigated whether intrastriatal estrogens or a catecholestrogen would have the same effect in castrated male rats.

Male and female Long-Evans hooded rats were gonadectomized and implanted with guide cannulae above the anterior dorsal striatum. After 2 wk recovery, independent groups of rats were implanted in the dorsal striatum with cannulae filled with 17αestradiol, 17βestradiol, 4-hydroxyestradiol, or cholesterol 4 hr prior to testing. Rats were tested for the DIR for 3 trials with a 5 min cutoff and a 30 sec intertrial interval. In both males and females this response duration was significantly increased by both isomers of estradiol. However, only in the males did the catecholestrogen significantly increase the DIR.

Thus the striatum of male and female rats responds similarly to estradiol, but only the male striatum is sensitive to the effect of a catecholestrogen on this behavior. The effect of catecholestrogens on DA metabolism may differ in male and female rats.

408.8

POTENTIATION OF THE DORSAL IMMOBILITY RESPONSE BY HORMONES IN THE BASAL GANGLIA OF OVARIECTOMIZED RATS. M.E. Meyer,* G.A. Cottrell and C. Van Hartesveldt. Psychology Dept., Univ. Florida, Gainesville, FL 32611.

The dorsal immobility response (DIR) is a species-typical inhibitory response that is elicited by grasping the rat by the dorsal skin at the nape of the neck and lifting it off its feet. The DIR persists until the rat emits escape-like behavior. We administered hormones directly to the basal ganglia of OVX female rats to determine their specificity and site of action on this response.

Long-Evans female hooded rats were OVX, then implanted with guide cannulae directly above the anterior dorsal striatum. Two wk after surgery, injection cannulae were filled with 17βestradiol, 2-hydroxyestradiol, progesterone, or cholesterol and bilaterally implanted for 4 hr into the dorsal striatum. Each rat was tested for the DIR for 3 trials with a 5 min cutoff and a 30 sec intertrial interval. After 1 wk recovery periods, each rat was tested with each substance in a Latin square design. Only the 17βestradiol significantly increased the duration of the DIR.

In a second experiment 17βestradiol was implanted in OVX females as above for 4 hr into the dorsal striatum, nucleus accumbens, globus pallidus, or cerebral cortex. The DIR was significantly increased with implants in the dorsal striatum and nucleus accumbens.

408.9

EFFECTS OF INTRASTRIATAL ESTRADIOL ON APOMORPHINE-INDUCED STEREOTYPED BEHAVIORS. R.L. Smith and C. Van Hartesveldt. Psychology Dept., Univ. Florida, Gainesville, FL. 32611.

Previous research has shown that estradiol can alter DA-related stereotyped behaviors. This study investigated whether direct application of estradiol to several regions of the basal ganglia could differentially affect individual apomorphine (APO)-induced stereotyped behaviors.

Female Long-Evans hooded rats were ovariectomized and bilaterally implanted with guide cannulae into the anterior-dorsal striatum, nucleus accumbens or globus pallidus. Two weeks after surgery animals were implanted with either 17αestradiol or 17βestradiol, and tested 48 hours later with 0.45 mg/kg apomorphine. One week later the animals were given the reverse treatment and tested with apomorphine again. Individual stereotyped behaviors were recorded every 5 sec. for one hour.

The 17βestradiol implants in the anterior dorsal striatum and globus pallidus decreased oral behavior during portions of the test session and increased sniffing behavior. Other implants were ineffective. Therefore, the effects of estradiol on APO-induced stereotyped behaviors are specific to the estradiol stereoisomer, site of implant and the behavior.

408.10

NUCLEUS OF THE POSTERIOR COMMISSURE IN THE RED EARED TURTLE, PSEUDEMYS SCRIPTA. P. M. Bell and P. S. Ulinski, Dept. Anatomy, University of Chicago, Chicago, IL 60637.

Like the substantia nigra, the nucleus of the posterior commissure (nPC) is a link between the striatum and tectum of reptiles. We have analyzed the functional architecture of nPC to help understand its role in motor behavior. nPC extends from behind the habenula, rostrally, to the rostral pole of the tectum, caudally. It comprises a cell plate just lateral to the central grey and a cell poor zone that extends laterally to nucleus pretectalis. The cell plate contains a dorsal field of small cells with round somata and a ventral field of larger cells with fusiform somata. In Golgi material, the small celled field contains bipolar cells with dorsoventrally oriented dendrites. The ventral field contains: (1) bipolar cells; (2) "rabbit" cells whose dendrites are usually oriented laterally, like ears, away from round somata; and (3) multipolar cells whose dendrites extend in many directions. The cells have smooth dendrites that extend up to one mm from their somata in the transverse plane. HRP injections in tectum retrogradely label only cells in the ventral field, but all three types are filled. Rabbit cells are situated primarily along the medial axis of the nucleus.

408.11

THE SUBSTANTIA NIGRA OF THE RED-EARED TURTLE, PSEUDEMYS SCRIPTA ELEGANS. A. F. Chang* and P.S. Ulinski. Dept. of Anatomy, University of Chicago, Chicago, IL 60637.

The striatum of turtles projects to the substantia nigra, which projects in turn to the optic tectum. The substantia nigra gives rise to a dopaminergic projection back to the striatum, but nothing is known of its functional architecture. Thus, tyrosine hydroxylase immunohistochemistry and Golgi and Nissl preparations were used to examine the morphology of cells in the substantia nigra of turtles. The substantia nigra is divided into two zones, the pars compacta and the pars reticulata, and is situated dorsolateral to the ventral tegmental area. Cells in pars compacta have thick dendritic trunks that arise from fusiform somata and bear two to four primary branches that are smooth and branch infrequently. They resemble cells stained in tyrosine hydroxylase immunohisto-chemical preparations. The orientation of pars compacta cells varies with their position. The most ventromedial have dendrites that run ventrolaterally while those in more dorsal regions have dendrites oriented progressively more mediolaterally. Those near the external border of the pars compacta send dendrites a short distance into the pars reticulata. Cells of the pars reticulata are smaller and have dendrites with a lateral-dorsal orientation. Cells in the ventral tegmental area are fusiform with dorsoventrally oriented dendrites and also stain with antisera against tyrosine hydroxylase. The dendritic fields of cells in all regions are flat in the tranverse plane.

408.12

CALCITONIN GENE-RELATED PEPTIDE (CGRP) IMMUNOREACTIVE AXONS IN THE RAT GLOBUS PALLIDUS AND SUBSTANTIA INNOMINATA: A LIGHT AND ELECTRON MICROSCOPIC STUDY.

H. Kuo* and H.T. Chang (SPON: R.C. Foehring) Department of Anatomy & Neurobiology, The University of Tennessee, Memphis, 875 Monroe Ave., Memphis, TN 38163.

Calcitonin gene-related peptide (CGRP) has been found within both axons and cell bodies in various parts of the mammalian central nervous system. In this study, the distribution of CGRP immunoreactive (CGRP+) axons in the rat globus pallidus (GP) and substantia innominata (SI) was investigated at both light and electron microscopic levels using a rabbit anti-CGRP antiserum generously provided by Dr. C. Sternini (Dept Medicine, UCLA). CGRP+ axons ramified extensively within SI. Bundles of CGRP+ axons and terminals were also found in discrete regions in the caudal and medial parts of GP bordering the internal capsule. The distribution of CGRP+ fibers appeared to overlap partially with that of the cholinergic neurons in GP and SI. Preliminary electron microscopic analysis revealed that CGRP+ boutons formed mainly asymmetrical synapses with dendrites of GP and SI neurons. Dendrites which were postsynaptic to CGRP+ boutons also formed many symmetrical synapses with unlabeled axon terminals. Since previous studies have shown that basal forebrain cholinergic neurons formed few synapses, the present result suggests that CGRP+ axons innervate mainly non-cholinergic GP and SI neurons. (This study was supported by USPHS Grants NS21003 and AG05944)

408.13

SPINDLE ACTIVITY IN THE THALAMUS IN VITRO.
M.Serafin* and M.Mühlethaler, Dept de Physiologie CMU, 1211 Genève 4, Switzerland.

Intracellular recordings from thalamic neurons have been obtained so far in situ in the cat and in guinea pig slices. However in order to study membrane properties within a complex circuitry alternative preparations must be used. We therefore recorded from thalamic neurons in an isolated whole brain preparation(IWB) and found that they displayed all the properties already described in slices. However they displayed in addition spindle activity either spontaneously or in the presence of barbiturates. These spindles were very much the same than those recorded in the intact cat. They were indeed characterized by a spindle duration of 1-3 sec. consisting of low threshold rebounds and spikes and the interspindle interval was 10-20 sec. These spindles could be manipulated either by brainstem stimulations or by the application of drugs in the perfusion.Our studies indicate that thalamic circuits are preserved in the IWB which could thus be used for studying intrinsic thalamic mechanisms as well as brainstem-thalamic interractions.
(Supported by a Swiss NSF grant no 3.288-0.85)

408.14

ELECTROPHYSIOLOGY OF ENTOPEDUNCULAR NEURONS AND THEIR RESPONSES TO SUBTHALAMIC STIMULATION IN RAT BRAIN SLICE PREPARATIONS.
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It is well demonstrated that single subthalamic (STH) neurons in the rat project both to the pallidum and to the substantia nigra (SN). Our previous *in vitro* study demonstrated that stimulation of the STH evoked monosynaptic EPSPs in SN neurons. In this study, we examined electrophysiology of entopeduncular (EP) neurons in the rat brain slice preparations.

Sagittal slices (thickness 400 μm) containing EP and STH were placed in a recording chamber. EP neurons were intracellularly recorded through glass microelectrodes filled with K-methylsulfate. STH was electrically stimulated through bipolar metal electrodes. EP neurons were classified into two groups based on their membrane properties. Type-I neurons, the major group, were characterized by having spontaneous repetitive firing and relatively short (less than 100 ms) spike after-hyperpolarization. Type-II neurons, in contrast, had no spontaneous firing, and the spike triggered by intracellular stimulation was followed by a long (more than 200 ms) after-hyperpolarization. In type-I neurons, stimulation of the STH evoked short latency monosynaptic EPSPs and bicuculline-sensitive monosynaptic IPSPs. In the type-II neurons, the STH stimulation evoked only EPSPs. Based on already known physiological and anatomical evidences, it is likely that these monosynaptic EPSPs originated from the STH. Supported by NIH grants NS 20702 to STK and NS 25783 to HK.

408.15

THE SUBSTANTIA NIGRA PARS RETICULATA PROJECTION TO THE SUBTHALAMIC NUCLEUS OF THE RAT.
H. Kita and S.T. Kitai, Dept. of Anatomy and Neurobiol., College of Medicine, The University of Tennessee, Memphis, Memphis, Tennessee 38163.

Projection from the substantia nigra pars reticulata (SNR) to the subthalamic nucleus (STH) was studied in rats using the PHA-L anterograde tracing technique. PHA-L was iontophoretically injected into the SNR. After two weeks of survival, animals were fixed and the brains sectioned on a vibratome. Sections were immunoreacted for PHA-L using the conventional ABC method (see J. Comp. Neurol. 260: 435-452, 1987 for more details of the technique). Injection of PHA-L into the SNR resulted in the labeling of a large number of boutons in the STH. Light microscopic analysis indicated that the size of PHA-L labeled boutons (about 1μm) is much larger than the tyrosine hydroxylase-immunoreactive (i.e., monoamine containing) boutons seen in this area. Electron microscopic analysis of the labeled terminals in the STH revealed that the labeled boutons were large and contained many spherical or ellipsoidal vesicles and a number of mitochondria. Most of them formed symmetrical synapses with dendritic shafts. These morphological features were very similar to the GAD-immunoreactive boutons in the STH. The observations indicated an existence of probably GABAergic projections from the SNR to the STH. This newly found projection may function as a negative feedback circuit of previously demonstrated excitatory STH projection to the SNR (Nakanishi et al., Brain Res.). Supported by NIH grants NS 20702 and NS 23886 to STK and NS 25783 to HK.

408.16

VOLTAGE-CLAMP ANALYSIS OF A TRANSIENT POTASSIUM CURRENT IN RAT NEOSTRIATAL NEURONS.
D.J.Surmeier, Jose Bargas* and S.T.Kitai, Dept. of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis,TN 38163.

Recent current-clamp studies have suggested that an A-like potassium current is present in rat neostriatal neurons (Galarraga et al., *Neurosci.Abstr.* 11:202,1985). In addition to its importance in a general model of neostriatal discharge, the characterization of an A-like current in neostriatal neurons may be of crucial importance to an understanding of the actions of neuromodulators, such as dopamine.

To this end, whole-cell voltage clamp recordings were made from rat neostriatal neurons grown in primary monolayer cultures. Cultures were derived from the striata of E17 rat embryos and grown in a basal medium (F12:DMEM) supplemented with serum (10%), insulin (5μg/ml), HEPES (3.574 gm/ml) and antibiotics. Cells were maintained in a humid, 5% CO2, 37 C environment. Recordings were made using conventional techniques. The normal bath solution contained (in mM): 120 NaCl, 6 KCl, 2 CaCl2, 1 MgCl2, 10 Na-HEPES, 6 glucose, pH=7.4, 300 mOsm. The intracellular solution contained (in mM): 98 K-gluconate, 20 TEA-Cl, 10 HEPES, 2 MgCl2, 2 ATP, 0.2 GTP, 5 EGTA, pH=7.2, 300 mOsm. Na currents were blocked by including 1-2 μM TTX in the bath.

All neostriatal neurons exhibited a transient outward current (N=41). In most neurons, the activation and inactivation characteristics of this outward current were similar to those described in other mammalian neurons. The current was substantially reduced by bath application of 2-5 mM 4-aminopyridine (N=6), a concentration which did not significantly reduce the sustained outward currents. The current was not blocked by bath application of inorganic Ca channel blockers Cd (up to 500 μM, N=8), Co (3 mM,N=4) or Mn (2 mM,N=4). An analysis of tail current reversals suggested that potassium was the principal charge carrier. Thus, the voltage-dependence, pharmacology and selectivity of this transient conductance suggests that it is similar to the A-channel conductance. Supported by NIH grants NS 20702 and NS23886 to STK and a Huntington's Disease Foundation grant to DJS.

408.17

QUINOLINATE AND KAINATE NEUROTOXICITY IN NEOSTRIATAL CULTURES IS POTENTIATED BY CO-CULTURING WITH NEOCORTICAL NEURONS.
E. Galarraga*,D.J. Surmeier and S.T. Kitai, (SPON: T.Gardiner). Dept. of Anatomy and Neurobiology, College of Medicine. The University of Tennessee, Memphis, Memphis, TN 38163.

There is a substantial loss in glutamate neurotoxicity in the neostriatum following cortical deafferentation (Biziere and Coyle, *Neurosci.Lett.* 8:303-310,1978). However, the role of cortical innervation in determining the relative sensitivity of different striatal phenotypes to glutamate excitotoxins is unclear. Because of the need to precisely control agonist concentrations and exposure time, a primary monolayer culture model of neostriatum and neocortex was used to address this role.

Cultures of embryonic (E17) rat neostriatum and neocortex were maintained with serum supplemented F12:DMEM basal media in a humid, 5% CO2, 37 C environment. After 12 days *in vitro*, cultures were exposed to concentrations of quinolinate (QUIN) or kainate (KA) ranging from 1 to 1000 uM for 5 mins. Cell counts were made from representative identified fields before and after (18-24 hr) the application of the toxins. Counts were expressed as a percentage of the number of cells originally present. In order to distinguish cortical from striatal neurons in co-cultures, cortical neurons were labeled with the fluorescent carbocyanine dye (DiI) during dissociation. Cortical neurons could be readily identified for several weeks with this procedure.

Our results indicate that QUIN and KA are neurotoxic to striatal neurons cultured in the absence of cortex. However, the neurotoxicity of both substances to neostriatal neurons was potentiated when they were co-cultured with neocortical neurons. D-2-amino-5-phosphonovalerate substantially reduced the neurotoxicity of QUIN but it failed to significantly reduce the effects of KA, suggesting that different mechanisms are responsible for the toxicity of these glutamate analogs. Preliminary immunocytochemical studies indicate that different neuronal phenotypes are affected by QUIN and KA as well.. Supported by NIH grants NS 20702 and NS23886 to STK and a Huntington's Disease Foundation grant to DJS.

408.18

MUSCARINIC RECEPTORS IN PRIMARY CULTURES OF RAT NEOSTRIATUM.
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We have characterized some pharmacological properties of the receptors expressed on intact striatal neurons. Cultures were generated from fetuses at 17 days gestation. After dissection and mechanical dissociation, cells were plated into 12 well dishes at 2000/mm^2 for neuronal cultures or 200/mm^2 for glial cultures. Cultures were fed serum-supplemented DME/F12 and maintained 13-14 days in vitro.

Binding studies were carried out in HEPES buffered Hank's balanced salt solution(HBSS) at 23 degrees C. Cultures were preincubated for 15 min. Steady state binding was achieved in 45 min. for [3H]N-methylscopolamine(NMS) at concentrations of 0.01 to 2 nM. Cultures were washed with ice-cold HBSS, scraped, and counted in Optifluor at 36% efficiency. Low levels of specific binding were measured in glial cultures, constituting less than 5% of that in neuronal cultures at equivalent NMS concentrations. Saturation data(n=5) at 12 concentrations(0.01 to 2 nM) of NMS were analyzed using the computer program LIGAND. The binding was well-characterized by a single site with a Kd of 89 ± 10 s.e.m. pM, linear Scatchard plots, and Hill plots with an average slope of 1.04 ± .05. Mean Bmax values were 10.9 ± 1.5 fmol/well, 187 ± 43 fmol/mg protein, or 32,000 ± 3000 sites/neuron. Displacement studies with pirenzepine (Boehringer Ingelheim) and carbachol using 0.1 nM NMS have EC_{50}'s of 201 nM and 72 μM, and Hill coefficients of 0.58 and 0.65, respectively. These results suggest multiple subtypes of the muscarinic receptor are expressed by striatal neurons. (Supported by U.S.P.H.S. Grants NS 20702 and NS 23886).

408.19

MEMBRANE PROPERTIES OF PEDUNCULOPONTINE NEURONS AND THEIR RESPONSES TO NIGRAL STIMULATION IN AN IN VITRO SLICE PREPARATION. Y.N.Kang*, S. Afsharpour, and S.T.Kitai. Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis,TN 38163.

Electrical membrane properties of neurons in rat pedunclopontine nucleus (PPN) and their responses to substantia nigra reticulata (SNr) stimulation were studied in parasagital brain slices (400-500 μm) which contained the brachium conjunctivum (BC), substantia nigra and the subthalamic nucleus (STH). Intracellular potentials were recorded using conventional techniques. SNr was stimulated by three bipolar stimulating electrodes. In someexperiments, a pair of additional stimulating electrodes was placed in STH or between STH and SNr. Twenty out of 80 recorded neurons were intracellularly injected with biocytin. Slices were fixed with 4% paraformaldehyde, immersed in 20% sucrose, frozen and sectioned at 50 μm and reacted with avidin-Texas Red for flourescent visualization. These sections were then processed immunocytochemically for ChAT or counter-stained with cresyl violet. All the injected neurons were located in PPN.

Electrical membrane properties of PPN neurons were studied by intracellular current injection through the recording microelectrode. The results indicated that PPN neurons may be classified into several types based on their electricalmembrane properties such as prominent afterhyperpolarization, TEA sensitive outward currents, TTX or cobalt sensitive slow inward currents and high threshold TTX insensitive spikes which were bloked by cobalt.

Responses to SNr stimulation were predominantly monosynaptic IPSPs. It was frequently observed that IPSPs were followed by rebound Na spikes possibly generated by two types of slow inward currents. Occasional EPSPs seen following SNr stimulation were considered to be due to the activation of passing fibers in SNr since stimulation of STH or the area between STH and SNr produced EPSPs with similar shape but with slightly longer latencies. Supported by NIH grants NS 20702 and NS 23886 to STK.

408.20

ELECTROPHYSIOLOGICAL AND DOUBLE-LABELLING IMMUNOHISTOCHEMICAL ANALYSES OF NEURONS IN THE SUBSTANTIA NIGRA ZONA COMPACTA OF THE RAT. J. Bargas*, E. Galarraga* , H. T. Chang and S. T. Kitai Department of Anatomy and Neurobiology, College of Medicine. The University of Tennessee, Memphis, Memphis, TN 38163.

Electrical membrane responses of the neurons in rat substantia nigra zona compacta (SNc) were studied in the *in vitro* slice preparation. Glass microelectrodes of DC resistances around 80-120 Megs filled with either K-acetate 3M or biocytin 4% and K-acetate 2M were used. Saline compositon was, in mM: NaCl 125, KCl 1.25, KH2PO4 1.75, CaCl2 2, MgCl2 1, HCO3Na 25, glucose 11. Temperature was between 34 and 36 C and osmolarity around 300 mOsM/l. Either coronal or sagital mesencephalic slices were used. The electrophysiology of the SNc has revealed substantial heterogeneity. A majority of SNc neurons had spontaneous low frequency firing of 0.5-9Hz. Input resistance (Rn) was strongly voltage dependent largely due to a strong time dependent anomalous rectification. Rn was 147±24 Megs (n=10) when measured at a holding potential near the spontaneous firing level (-55 mV). Depolarizing currents given at -60 mV evoked repetitive firing. However, if the same depolarizing currents were given at -80 mV, many neurons exhibited low threshold spikes (LTS) of variable amplitude during the test depolarization. The LTS could elicit a single spike or a burst of fast spikes followed by a strong post-burst hyperpolarization (PBH). In some instances, oscillatory bursting behavior (2-4Hz) was seen. This depended on the holding membrane potential and the magnitude of the depolarizations as well as on the PBH amplitude. Neurons which were intracellularly injected with biocytin had their morphology revealed by reacting with avidin conjugated to Texas Red. Subsequently, the tissue was processed for tyrosine hydroxylase immunoreactivity with secondary antibodies conjugated to FITC. Preliminary results indicate that double-labelled biocytin and TH+ neurons can be readily demonstrated with this procedure. Results also suggest that there is a significant probability of impaling non-dopaminergic neurons in the SNc. Supported by NIH grants NS20702 and NS23886 to STK

408.21

PATTERNS OF TERMINATION OF RAT BASAL GANGLIA AND CEREBELLAR EFFERENTS IN THE THALAMUS: STRICTLY SEGREGATED AND PARTIALLY OVERLAPPED PROJECTIONS. . J.M. Deniau*, H. Kita & S.T. Kitai (SPON. D. Desiderio). Dept. of Anatomy & Neurobiol. , College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163. *Lab. de Physiol. des Centres Nerveux, Univ. Pierre et Marie Curie, F-75230 Paris Cedex 05, France

There is a widely held view that basal ganglia and cerebellar outputs act independently via separate subcortical channels. In this study, the pattern of innervations provided by the deep cerebellar nuclei (CDN), entopeduncular nucleus, and the substantia nigra pars reticulata was reinvestigated in rats using the PHA-L anterograde tracing technique.

PHA-L was iontophoretically injected into these brain areas. After two weeks of survival, animals were perfuse fixed and the brains sectioned on a vibratome. Sections were immunoreacted for PHA-L using the conventional ABC method.

Light microscopic observation indicated that, in the ventrolateral and the external ring of the intralaminar thalamic nuclei, basal ganglia and CDN efferents are strictly segregated. CDN fibers in these areas were either thick or thin and formed high density of en passant and terminal boutons. In contrast, in the ventromedial and parafascicular thalamic nuclei and in the deep tectum, basal ganglia and CDN efferent sites overlap. CDN fibers in these areas were usually thin and formed relatively small en passant and terminal boutons. These observations suggest that in the rat the cerebellum and the basal ganglia have, on one hand, their own distinct pathways through the thalamus; and on the other hand, they may share some thalamic and tectal neurons in processing their information. Supported by NIH grants NS 20702 and NS 23886 to STK and NS 25783 to HK.

BASAL GANGLIA AND THALAMUS: MOTOR SYSTEMS V

409.1

PERINATAL COMPETITION BETWEEN IPSI- AND CONTRALATERAL PROJECTING NIGROSTRIATAL NEURONS ELIMINATES CONTRALATERAL PROJECTING CELLS. G. Fishell, M. Takada, T. Hattori, and D. van der Kooy. Neurobiology Research Group, Dept. of Anatomy, University of Toronto, Toronto, Canada M5S 1A8

By embryonic day 16 in the rat, separate cells in the substantia nigra have developed ipsi- and contralateral axonal projections to the striatum. Retrograde fluorescent tracer (Fast Blue (FB)) injections into the striatum during the late embryonic and early postnatal period, revealed that both the ipsi- and contralateral nigrostriatal projections undergo marked periods of perinatal cell death. However, the time courses of cell death in these two populations of dopaminergic substantia nigra neurons are surprisingly different. By postnatal day 1, 85% of the embryonic population of contralateral projecting nigrostriatal neurons die. In contrast, the number of striatal projecting neurons in the much larger ipsilateral pathway increases over the same time period, peaking in size at postnatal day 4. By postnatal day 7, cell death reduces the ipsilateral pathway by 25%, to its adult size. Comparison of short versus long term retrograde labeling of the ipsi- and contralateral pathways revealed that cell death rather than axon retraction is responsible for the postnatal decrease in both pathways. In order to test whether ipsi- and contralateral projecting nigrostriatal neurons compete for survival, we lesioned the ipsilateral nigrostriatal projection on embryonic day 19. Unilateral knife cuts were made in the ventral mesencephalon caudal to where the contralateral projection decussates. Lesioned animals received a striatal injection of FB ipsilateral to the lesion, one day prior to their sacrifice on postnatal day 7. The striatum on the lesioned side was approximately 60% of the volume of the contralateral control striatum. Success of the lesion was estimated by the reduction in the number of retrogradely labeled neurons in the ipsilateral substantia nigra. The embryonic lesions produced a 90% loss of ipsilateral nigrostriatal projections. However, the normal 85% cell death of contralateral projecting neurons was completely abrogated. These results suggest that competition between ipsi- and contralateral projection neurons is the mechanism that eliminates much of the transient contralateral projecting nigrostriatal pathway.

409.2

ULTRASTRUCTURAL CHANGES IN CAUDATE NEUROPIL FOLLOWING QUINOLINIC ACID LESIONS. R.C. Roberts and M. DiFiglia. Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

In order to determine the anatomical basis of excitotoxicity in the caudate nucleus as a model of Huntington's disease (HD), the effects of injections of quinolinic acid (20ug in 0.6ul) into adult rat striatum were investigated at the light and electron microscopic level at 2 (n=4), 7 (n=2) and 30 (n=3) weeks post lesion. Neuronal and synaptic density were examined at each time point in four regions: 1) lesion (intact tissue exhibiting profound neuronal loss), 2) proximal transition ("PT"=first 125um from the edge of the lesion, 3) distal transition ("DT"=500-650um from the edge of the lesion) and 4) contralateral, uninjected side.

Neuronal vs Synaptic Density

wks post L		2	7	30
zone				
L	neurons	3*	2*	1*
	synapses	10*	15*	63
PT	neurons	52*	60*	74*
	synapses	78	85	97
DT	neurons	89*	92	98
	synapses	89	104	101

values expressed as % of control
* p<.025

Both neuronal and synaptic density were markedly reduced in the lesion zone at all time points and in the PT at 2 and 7 wks. Relative to control, synaptic density was greater than neuronal density in the lesion (7-63%) and PT zones (approx. 25%) at all time points, which may reflect a compensatory regenerative response. In the lesion zone large neurons comprised 35-55% of total neurons as compared to 2-3% in the PT zone and 1% in controls. In the lesion zone, the large neurons retained their normal ultrastructure and synaptic inputs despite the severe loss of neuronal integrity in the surrounding area. Results suggest that excitotoxic lesions cause both degenerative and regenerative changes in caudate neuropil and may parallel findings made in this laboratory in Golgi impregnated caudate tissue from HD brains. Supported by NIH grant NS-16367 to MD, a Huntington's Disease Society of America grant to RCR & a NINCDS postdoctoral grant to RCR.

409.3

SPECIFIC STRIATAL LESIONS INFLUENCE BETA, BETA - IMINODIPROPIONITRILE-INDUCED DYSKINESIAS. B.I. Diamond, R. Borison, H. Nguyen.* Department of Psychiatry, Medical College of Georgia.,Augusta, GA 30917.

It has been well documented that the administration of beta, beta-iminodipropionitrile (IDPN) to various animal species produces a hyperkinetic movement disorder resembling phasic dystonia. It was the aim of this study to investigate the role of the striatum in regulating IDPN-induced movements. Male Sprague Dawley rats (200grams) were anesthetized with pentobarbital (40 mg/kg;i.p) and stereotaxic kainic acid (3nM) lesions were placed in either the dorsal or ventral caudate nucleus. Control animals received phosphate buffer striatal injections. After 1 week animals received two daily injections of IDPN (100mg/kg;i.p) and were rated 1 week later. IDPN produced circling, retropulsion and head and neck dystonia in rats. These movements were antagonized but not abolished by ventral striatal kainic acid lesions. In contrast dorsal striatal lesions with kainic acid markedly exacerbated these hyperkinesias. Other lesioned areas of the brain did not markedly affect IDPN-induced hyperkinesias. These results suggest that the striatum modulates the movements induced by IDPN but is not the initiating site for its action.

409.4

CEREBELLAR PURKINJE CELL: ANOTHER NEURONAL BASIS OF MPTP-INDUCED PARKINSONISM. M. Takada, T. Sugimoto and T. Hattori. Dept. of Anatomy, University of Toronto, Toronto Ontario M5S 1A8, Canada. Dept. of Anatomy, Kansai Med. Univ., Moriguchi, Osaka 570, Japan.

In order to re-examine neuronal bases of MPTP-induced parkinsonism, MPTP (30 mg/kg b.w./i.p. injection) was given to mice (1-10 daily injections). One to three weeks after the last injection, nicely-fixed brain tissue was Nissl-stained and compared with control normal sections. Tremendous cell loss and degeneration were observed in the substantia nigra pars compacta. The only other brain area that showed similar marked morphological changes, was the Purkinje cell (PC) layer of the cerebellum. Especially in the flocculus/paraflocculus, more than 50% of PC exhibited cell loss and various stages of cell degeneration. We have also found that the C57 black mouse is more susceptible than the CD1 white mouse to MPTP. Moreover, in the flocculus/paraflocculus of the rat given the intraventricular or intracisternal injection of colchicine, a considerable number of PC were immunoreactive for tyrosine hydroxylase (TH), but not dopamine-beta-hydroxylase. No other cellular elements displayed TH immunoreactivity in the cerebellum. These data strongly suggest that the PC may provide another neuronal basis of MPTP-induced motor impairments.

Supported by the Medical Research Council of Canada.

409.5

STRIATAL DOPAMINE AND THE INTERFACE BETWEEN ORIENTING AND INGESTIVE FUNCTIONS. S. Hall* and T. Schallert. (SPON: E. Bigler). Department of Psychology and Institute for Neurological Science, University of Texas at Austin, Austin, TX 78712.

Recent experiments have implicated forebrain catecholaminergic projections in a unique switching mechanism that enables sensory orientation to occur during ongoing feeding behavior. Sensory-related cells identified in the striatum (Schneider et al., *Neurophysiol.*, 1985) may serve as part of a system that redirects attention away from ingestive behavior and toward external stimulation (Schallert & Hall, *Behav. Brain Res.*, 1988; Hall & Schallert, *Brain Res. Bull.*, in press). In the present study, 6-hydroxydopamine (6-OHDA, which selectively destroys dopaminergic neurons when followed by a noradrenergic uptake inhibitor) or ibotenic acid (an axon-sparing neurotoxin) were infused directly and unilaterally into the ventrolateral striatum in rats. In addition to a battery of sensory and motor tests, the animals were examined for their reactions to tactile stimulation of the vibrissae during or in the absence of eating. During non-eating trials, orienting was rapid and reliable to stimuli presented on either side of the body midline. While eating, contralateral orienting never occurred in the 6-OHDA treated rats, even when the stimulation was intense, whereas ipsilateral orienting was unaffected. In contrast, the "disengage deficit" did not occur in animals treated with ibotenic acid. In other sensorimotor tests, ibotenic acid yielded mild and/or very transient asymmetries, whereas 6-OHDA yielded severe and chronic asymmetries. It was suggested that the capacity to disengage from ingestive behavior may depend importantly and specifically on the integrity of dopaminergic input to the striatum. Supported by NS-23964.

409.6

BOTH NICOTINIC AND MUSCARINIC RECEPTORS MEDIATE THE EXCITATORY ACTIONS OF ACETYLCHOLINE ON DOPAMINERGIC A9 AND A10 NEURONS. M.G.Lacey, P.Calabresi & R.A.North. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Intracellular recordings were made from presumed dopaminergic rat midbrain neurons in a slice preparation. Acetylcholine, applied by superfusion or by pressure ejection, caused a depolarization or an inward current which was potentiated by neostigmine (1 μM). The response comprised a large transient component of rapid onset and a smaller, more slowly developing component; both were unaffected by tetrodotoxin. The fast component increased in amplitude on hyperpolarization, was accompanied by an increase in membrane conductance, blocked by hexamethonium (100 μM) and cross-desensitized to nicotine (100 μM).

The slow component was mimicked by muscarine (1-30 μM) and blocked by scopolamine (3 μM). Muscarinic depolarizations and inward currents in the range -45 to -80 mV were generally associated with a conductance decrease. Muscarinic depolarizations and inward currents persisted at potentials negative to the K^+ equilibrium potential (extracellular K^+ concentration 2.5 to 20.5 mM). Dose-response curves to muscarine were shifted to the right by pirenzepine (30-300 nM), implicating an M_1 type receptor in this effect.

409.7

MODULATION OF ANTERIOR THALAMIC (AT) CELLULAR RESPONSIVENESS BY BRAINSTEM CHOLINERGIC (Ch) AFFERENTS. D. Paré, D. Bouhassira*, M. Deschênes and M. Steriade. Lab. Neurophysiol., Sch. of Med., Univ. Laval, Québec, Canada.

In contrast with most dorsal thalamic nuclei, AT cells are devoid of input from the reticular thalamic nucleus (J. Comp. Neurol. 1984, 229: 531-547). Thus, AT cells constitute a unique model to study inhibitory processes mediated by local-circuit cells and their modulation by the Ch afferents from the brainstem laterodorsal tegmental (LDT) nucleus. In order to study this problem, 86 AT neurons, physiologically identified by their short latency (3-5 ms) activation from the mammillary bodies (MB), were recorded extracellularly in the chronically-implanted, head-restrained cats during the sleep-waking cycle. The synaptic responsiveness of AT cells was assessed by comparing their response probability to two consecutive shocks (10 to 200 ms apart) applied to the MB or cingulate cortex.

Orthodromic activation of AT cells was always followed by a short (60-70 ms) and state-independent period of decreased responsiveness, presumably due to the activation of local inhibitory interneurons. The short duration of this inhibitory period contrasted with the much longer (150 ms) period of decreased responsiveness found in intralaminar neurons during slow-wave sleep. Short conditioning trains applied to the LDT nucleus increased the response probability of AT neurons to both shocks. LDT trains did not interfere with the inhibitory period induced by the first MB shock and, in some cases, increased it. No significant differences were observed when the same tests were carried out in reserpine-treated cats (0.5 mg/kg), thus suggesting that these effects were not dependent on coactivation of monoaminergic fibers. These results indicate that activation of Ch LDT cells can improve the ability of AT cells to relay incoming signals toward the cortex and preserve the local inhibitory processes required for analytic processing. Supported by MRC Grants MT-3689 and MT-5877.

409.8

DOPAMINE AND GABA ARE TRANSMITTERS IN THE NIGROTECTAL PATHWAY TO THE PERICENTRAL INFERIOR COLLICULUS. U.E. Olazábal[1] and J.K. Moore[2], Depts. of Psychology[1] and Anatomical Sciences[2], SUNY at Stony Brook, NY 11794.

Previous studies in this laboratory have demonstrated a direct projection from the substantia nigra, pars lateralis, (SN_L) to the rostrolateral pericentral zone of the inferior colliculus (IC_R). In addition, tyrosine hydroxylase (TH) and GABA immunohistochemical analyses have shown the presence of dopaminergic and GABAergic neurons in the SN_L. In order to determine the chemical specificity of this nigrotectal system, the present experiments combined TH and GABA immunohistochemistry with retrograde transport from the IC_R. Guinea pigs received small unilateral injections of a fluorescent tracer, either rhodamine-conjugated latex-microspheres or fluorogold, into the rostrolateral pericentral inferior colliculus. Tissue sections were processed for visualization of TH or GABA using the avidin-biotin variation of the PAP technique. Results indicated that approximately 70% of tracer-labelled SN_L neurons were GABA+, while about 15% of the total number of backfilled cells were TH+. Both types of double labelled neurons were multipolar cells, 20-30μm in diameter, with three to five large dendrites. Several lines of evidence suggest that the IC_R forms part of a sensorimotor pathway. The dopaminergic and GABAergic projections from the substantia nigra may thus exert a modulatory influence on acousticomotor behavior.

409.9

THE DISTRIBUTION OF THE CEREBELLOTHALAMIC AND NIGROTHALAMIC PROJECTIONS IN THE DOG. S. T. Sakai, K. Patton* and A. Smith*, Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

A series of horseradish peroxidase (HRP) injections were made into the deep cerebellar nuclei. At the same time, tritiated amino acid injections were made into the contralateral substantia nigra in anesthetized dogs. Following survival times of 2-4 days, the brains were aldehyde fixed and processed for both HRP histochemistry and autoradiography. The cerebellothalamic projections are widespread and bilateral, filling a wide arc including the rostral and dorsal portion of the ventral lateral nucleus (VLd), VL proper and the ventromedial nucleus (VM). In contrast, the nigral efferents were more limited in distribution labeling the ventral anterior nucleus (VA), the medial portion of VL and VM. Although the double labeled regions were closely opposed within VL, careful analysis of the double stained sections revealed that the patches of nigral silver grains did not directly coincide with the HRP positive patches from the cerebellum. However, a number of HRP labeled fibers were observed in close proximity to the nigra silver grains within VM. These data suggest that the motor thalamus may be primarily involved in the parallel processing of cerebellar and nigral information. (Supported by N.I.H. Grant NS18851 and B.R.S.G. funds to the College of Human Medicine, M.S.U.)

409.10

THE ORGANIZATION OF NIGROSTRIATAL PROJECTIONS IN THE DOG. D. Tanaka, Jr., S. T. Sakai and A. Smith*, Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Little is known regarding the detailed topography of the nigrostriatal system in the dog. In the present study, the autoradiographic method was systematically used in order to trace these projections. A series of pressure injections of tritiated amino acids was made into the rostral, caudal, lateral and medial parts of the substantia nigra (SN) in anesthetized dogs. After survival times of 3-7 days, the animals were perfused with formalin-saline and the brains processed for standard autoradiography. Nigrostriatal projections terminated in a widespread patch-like pattern in the ipsilateral striatum (St). A topographical relationship emerged whereby obliquely oriented longitudinal slabs of the St received a rostrocaudal and mediolateral inverted nigral input. The lateral portion of SN projects to the ventromedial part of the St while the medial part of SN projects to the dorsolateral St. Injections confined to the rostral half of SN primarily labeled the caudal part of St. These results suggest that the topographical organization of the canine nigrostriatal system may be more complex than that previously described in other species. (Supported by N.I.H. Grants NS16991 and NS18551 and B.R.S.G. funds to the Colleges of Human Medicine and Veterinary Medicine, M.S.U.)

409.11

SUBSTANTIA NIGRA AFFERENTS OF THE NUCLEUS TEGMENTI PEDUNCULOPONTINUS IN THE RAT. B. Spann and I. Grofova, Dept. of Anatomy, Michigan State Univ., E. Lansing, MI 48824.

Previous studies have indicated that the substantia nigra, pars reticulata(SNr) is involved in the control of certain aspects of motor behavior and may mediate these effects through projections to the nucleus tegmenti pedunculopontinus(PPN). The present light and electron microscopic study utilizes the anterograde transport of lectin Phaseolus vulgaris(PHA-L) to delineate the origin, distribution and mode of termination of nigral fibers in the PPN.

Small and large injections of the tracer in different regions of the SNR resulted in similar distribution of the terminal fibers in the PPN. Both subdivisions, the subnucleus compactus(PPNc) and dissipatus(PPNd) appeared to receive the nigral afferents. However, the overall projection to the PPNd was far more prominent than that to the PPNc. A particularly dense plexus was seen in the medial PPNd in close approximation to the superior cerebellar peduncle. Although the terminal varicosities were often seen close to the somata of PPN neurons, the electron microscopic examination revealed mostly termination on the dendrites.

The observations suggest that the nigral afferents are likely to originate from the entire SNr and do not exhibit any distinct topographical organization. They terminate throughout the PPN and may impinge on dendrites of various populations of the PPN projection neurons. (Supported by N.I.H. Grant NS25744).

409.12

PROJECTIONS FROM THE NUCLEUS TEGMENTI PEDUNCULOPONTINUS TO THE BRAINSTEM RETICULAR NUCLEI. I. Grofova and B. Spann, Dept. of Anat., Mich. State Univ., E. Lansing, MI 48824.

Previous studies have indicated that the basal ganglia may control movement through a pathway involving the nucl. tegmenti pedunculopontinus(PPN). Since there exists only a meager projection from the PPN to the spinal cord, it is presumed that the PPN is linked to the spinal motor system through the reticular formation(RF) of the brainstem. This study utilizes the anterograde transport of lectin Phaseolus vulgaris(PHA-L) to delineate the distribution of PPN efferents in the pontine and medullary RF of the rat.

Following iontophoretic injections of PHA-L involving only the PPN, a significant proportion of labeled axons course caudally through the pontine and medullary RF. Within the nucl. reticularis pontis oralis and caudalis some of the axons collateralize to form a dense terminal plexus within these nuclei. Many labeled axons terminate within the medullary RF, particularly in the ventromedial portions of the nucl. reticularis gigantocellularis and its subdivisions(GiV, Giα, PGi), paramedian reticular nucleus and the ventral reticular nucleus of the medulla.

The results provide definite evidence of a substantial PPN projection to the pontine and medullary reticular nuclei containing reticulospinal neurons. They further support the notion that the PPN may serve as a relay between the basal ganglia and lower motor system. (Supported by N.I.H. Grant NS25744).

409.13

THE AMYGDALO-NIGRAL PROJECTION: AN ANTEROGRADE STUDY WITH PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L). C. Gonzales* and M.-F. Chesselet. (SPON: R. Harner) Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

As part of our research to determine the pattern of innervation of extrastriatal afferents to the substantia nigra, we injected the anterograde tracer PHA-L iontophoretically (5 uA, 7 sec. on, 7 sec. off for 15 min.) into the central nucleus of the amygdala. After survival times of 11-14 days, animals were perfused transcardially, the brains were removed and 30 um thick sections were taken. The presence of PHA-L was determined immunocytochemically in these sections using the avidin-biotin system with diaminobenzidine as the chromogen.

Injection sites that encompassed most of the central nucleus as well as discrete injections of the medial subregion resulted in immunoreactively labeled axons and boutons in the medial aspect of the substantia nigra pars compacta. Injection sites that involved either the medial or medial and lateral subregions but were confined to the ventral part of the nucleus produced some labeling in the lateral aspect of the pars compacta and few if any labeled processes in substantia nigra pars lateralis. In one case, extensive labeling of axons and boutons was seen in both pars lateralis and the lateral division of pars compacta when the injection site was in the most dorsal part of the central nucleus. Immunoreactivity was not seen in the substantia nigra pars reticulata after any of these injections. The results suggest that central nucleus of the amygdala projections to the substantia nigra are more extensive than previously reported. Furthermore, subregions of the central nucleus project to different parts of the substantia nigra suggesting an heterogenous organization of the pathway. Supported by BNS 86-07645 and the Dystonia Medical Research Foundation.

409.14

STRIOSOMAL DISTRIBUTION OF NEURONS EXPRESSING HIGH LEVELS OF PREPROTACHYKININ mRNA IN THE RAT STRIATUM. E. Robbins* and M.-F. Chesselet. (SPON: M. Lewis) Dept of Pharmacology, Med. Coll. Pennsylvania, Philadelphia PA 19129.

Previous studies have shown that striatal tachykinin neurons are heterogeneously distributed, with those in the striosomes expressing a higher level of substance P-like immunoreactivity (SP-LIR). In order to determine whether this pattern is related to differences in tachykinin gene expression, we examined the distribution of neurons containing the mRNA encoding beta-preprotachykinin (PPT) in the striatum. Sets of adjacent striatal sections were processed for in situ hybridization histochemistry using a 35S-RNA probe for PPT (Affolter, Zurich) and for 3H-naloxone binding. The location of cells labelled for PPT mRNA was compared to that of patches of dense 3H-naloxone binding sites which characterize the striosomes. The very large majority of PPT mRNA-positive cells observed in the striatum after short development times were located within the boundaries of the naloxone patches. Labelled cells were seen outside the patches only after longer development times, suggesting that these cells contained a lower level of PPT mRNA. The results show that the tachykinin cells of the striosomes express high levels of PPT mRNA, suggesting that the more intense SP-LIR observed in these neurons than in those of the matrix is related to a difference in tachykinin synthesis rather than storage or transport. Supported by BNS 86-16841 and the Commonwealth of Pennsylvania.

409.15

IMMUNOHISTOCHEMICAL DETECTION OF ENKEPHALIN AND SUBSTANCE P IN THE PALLIDUM AND SUBSTANTIA NIGRA OF THE AGED RAT. K. Yurko, E. Robbins* and M.-F. Chesselet, Dept of Pharmacology, The Med. Coll. Pennsylvania, Philadelphia, PA 19129.

Immunohistochemical studies in the brain of patients with adult onset Huntington's disease (HD) have revealed that enkephalin (ENK)-positive fibers in the external pallidum, and substance P (SP)-positive fibers in the substantia nigra pars compacta, were decreased at earlier stages of the disease than SP-positive fibers innervating the internal pallidum (Albin et al., Neurosci. Abs. '87, 1361). In order to determine whether a similar pattern of loss occurs during normal aging in the rat, we analyzed met-ENK- and SP-like immunoreactivity in sections of brain from Fisher 344 rats aged 4, 16 and 24 months. Indirect immunofluorescence immunohistochemistry still revealed a dense plexus of ENK-positive fibers in the globus pallidus, and of SP-positive fibers in both the substantia nigra and the entopeduncular nucleus in 24 month old rats. Similar ENK and SP immunostaining was also observed at all ages examined using a 125I-labelled secondary antibody and film autoradiography. Quantification of optical densities in the films using a DUMAS image analysis system revealed a slight decrease in SP-like immunoreactivity in both substantia nigra and entopeduncular nucleus in the aged rats. The results show that the pattern of alteration of striatal efferent neurons during normal aging in Fisher 344 rats does not parallel that seen in patients with HD. This further supports the hypothesis that the differential alteration of striatal efferent neurons observed during HD is related to a specific effect of the deficient gene. **Supported by BNS 86-16841.**

409.16

PHA-L STUDY OF THALAMOCORTICAL FIBERS OF THE CENTRAL LATERAL NUCLEUS. G. J. Royce. Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

The PHA-L lectin was injected into the central lateral (CL) nucleus in cats. The laminar distribution and morphology of labeled fibers was observed in the cortex. The most significant finding of this study is that labeled axons are present in layer I of several cortical regions. These axons are primarily oriented parallel to the cortical surface. Some long horizontal axon segments in layer I reach up to 1 mm in length. Labeled axons are also found in the deeper layers, especially layer III, where many are oriented radially. Wherever found, labeled axons have collaterals, varicosities, and terminal boutons. Some long axons traverse the cortex to reach layer I, and resemble the "unspecific or pluriareal" axons described by Lorente de No ('38).

The topographic distribution of the PHA-L labeled axons is similar to that found in our autoradiographic cases. Labeled axons are present in the lateral and suprasylvian gyri, and presylvian, cruciate, splenial, lateral and suprasylvian sulci; and limbic areas, including the cingular and retrosplenial areas.

Supported by NIH grant NS13453.

409.17

STUDIES ON DOPAMINERGIC PROJECTIONS ARISING IN THE DORSAL RAPHE. T. R. Stratford and D. Wirtshafter. Dept. Psych., Univ. of Illinois at Chicago, Chicago, Il. 60680.

While the serotonergic efferents of the dorsal raphe (DR) have been extensively investigated, little is known about the termination sites of non-serotonergic efferents. In the following study, adult male rats received 0.1 ul injections of the fluorescent retrograde tracer Fast Blue bilaterally into the substantia nigra (SN), the striatum (CPu) or the nucleus accumbens (Acb). Following a 5 day survival period, the brains were removed and processed for the immunocytochemical detection of tyrosine hydroxylase using a rhodamine-labeled secondary antibody. In each of the brains a population of tyrosine hydroxylase-immunoreactive (THI) neurons, apparently continuous with those in the caudal linear nucleus, was observed in the rostral aspect of the DR. Although a large number of retrogradely labeled cells projecting to the CPu or the SN were observed intermingled with the THI neurons, very few of the THI neurons were double-labeled following CPu injections and none following SN injections. In contrast, approximately 10% of the THI cells in the DR were double-labeled following injections confined to the Acb. The double-labeled cells appeared to be distributed uniformly throughout the THI cell population.

In conclusion, the projection pattern of THI cells in the DR appears to differ significantly from that of the serotonergic population and seems to resemble that of THI cells in the ventral tegmental area. (Supported by NS21350)

409.18

CHEMOARCHITECTONIC CHARACTERIZATION OF THE VENTROLATERAL AND OTHER VENTRAL TIER NUCLEI IN RAT THALAMUS. L.J. Sansone*, J.C. Hedreen, L.J. Martin*, D.L. Price, and M.R. DeLong (SPON: S.J. Herdman). Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182

To improve cyto- and chemoarchitectonic definition of ventral tier nuclei in rat thalamus, serial sections were stained for acetylcholinesterase (AChE), glutamic acid decarboxylase (GAD), cytochrome oxidase (CO), and Nissl. Ventrolateral (VL), ventromedial (VM), ventroposterolateral, ventroposteromedial, submedius, and posterior nuclei were examined. The VL complex was divisible into three zones: anterior-ventrolateral (VLA); intermediate (VLB); and caudal-dorsomedial (VLC). AChE innervation (pattern of stained fibers and puncta) was light in VLA and VLC and moderately dense in VLB. GAD innervation was generally light in VLC and denser in VLA and VLB. CO profiles were similar in all regions of VL. The VM nucleus displayed fine CO puncta (in contrast to coarser, more scattered, short fibers in VL), contained smaller neuronal perikarya than VL, exhibited AChE innervation resembling that of the adjoining VLC (except for a denser, curlicue pattern in the ventromedial third), and showed variable GAD patterns with a coarse, dark-staining plexus on the lateral border. The subdivision of VL into three chemoarchitectonically distinct regions suggests possible correspondnece with the distribution of afferent connections of VL and possible homologies with VL subdivisions in primates.

409.19

PHAL STUDIES OF THE EFFERENT CONNECTIONS OF RAT GLOBUS PALLIDUS. Wm. A. Staines, Department of Anatomy, University of Ottawa, Ottawa, ONT. K1H 8M5.

The projections of the globus pallidus of the rat were studies using anterograde tracing with Phaseolus vulgaris Leucoagglutinin (PHAL) in combination with retrograde fluorescent tracing methods and immunohistochemistry for neurotransmitter markers. Anaesthetized animals received iontophoretic injections of PHAL into various sites within the globus pallidus and were perfused at survival times of from 10 days to 2 weeks. Some animals received injections of Fluorogold into the thalamus, superior colliculus or midbrain reticular formation one day prior to perfusion.

Labelled pallidal efferent fibers and terminals were found within the caudate-putamen, reticular nucleus of the thalamus, entopeduncular nucleus, subthalamic nucleus and the substantia nigra. A single fiber morphology was found within all of these sites, consisting of large, regularly spaced varicosities en passant. A few fibers with similar morphology were found within restricted regions of the frontal cortex as well. Pallidal afferents to the substantia nigra showed a striking, perisomal clustering about the cell body and primary dendrites of GABAergic output neurons. A similar but less dramatic perisomal pattern of termination was seen around somatostatin-containing neurons of the caudate-putamen. (Supported by a grant from the MRC).

409.20

DISTRIBUTION OF CHOLINERGIC PERIKARYA WITH RESPECT TO HETEROGENEITIES IN SUBSTANCE P STAINING IN THE CAUDATE NUCLEUS OF THE CAT. M. Martone*, D. M. Armstrong, P. M. Groves. Univ. California San Diego, CA 92093.

Both substance P (SP) and choline acetyl transferase (ChAT) are known to be distributed heterogeneously in the mammalian striatum. In the present study, we examined the relationship between cholinergic perikarya and SP immunoreactivity in the caudate nucleus of adult cats using a double-label immunocytochemical protocol. Fifty micron sections were cut throughout the extent of the caudate nucleus and labeled sequentially for ChAT (antibody provided by L. Hersh) and SP (Sera Labs). To distinguish the two labels at the light microscopic level, nickel chloride ($NiCl_2$) was added as an intensifier to the diaminobenzidine (DAB) reaction for the ChAT antibody. This resulted in a purple reaction product that was clearly distinguishable from the light brown reaction product of the unintensified DAB reaction used to localize SP.

Preliminary observations suggest that ChAT-positive perikarya are more numerous in areas that also stain intensely for SP. Thus, in the dorsal striatum, ChAT-positive cell bodies appear to be associated with the SP-rich patches that are visible against a lighter staining matrix. In contrast, at more ventral levels, ChAT positive cells appear to avoid the SP-poor zones visible at this level and are more concentrated in the SP rich matrix.

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409.21

SUBSTANCE P AND NEUROKININ A STIMULATION OF NIGROSTRIATAL DOPAMINE AFTER INTRANIGRAL IBOTENIC ACID LESIONS. M. Reid*, M. Herrera-Marschitz*, T. Hökfelt*, U. Ungerstedt* (SPON: B. Meister). Dept. of Pharm. and Hist., Karolinska Institute, 104 01 Stockholm, Sweden.

The striatonigral pathway contains several neurotransmitters, two of which are considered to represent excitatory regulation of nigrostriatal dopamine (DA); substance P and neurokinin A. Local injection of substance P (0.00007-7.0 nmol/0.2 µL) or neurokinin A (0.009-9.0 nmol/0.2 µL) into the substantia nigra, pars reticulata (SNR) of halothane aneasthetized male Sprague-Dawley rats produced long lasting increases in ipsilateral striatal DA as measured by in vivo microdialysis. Intranigral injections were repeated in animals with ibotenic acid lesions (5.0 µg/0.5 µL) impairing non-dopaminergic neurons of the SNR (preliminary studies show only minor damage to the tyrosine hydroxylase positive dendrites in area of lesion). In the lesioned animals substance P (0.07 nmol/0.2 µL) stimulated striatal DA (avg. max. increase: 32.4 ± 5.9%, N=4), slightly less than in non-lesioned animals (53.3 ± 13.4%, N=7), while neurokinin A (0.09 nmol/0.2 µL) stimulated striatal DA (52.4 ± 11.2%, N=4) slightly more than in non-lesioned animals (36.9 ± 3.1%, N=5). These data indicate that striatonigral substance P and neurokinin A may stimulate nigrostriatal DA via monosynaptic input onto DA cells and/or dendrites in the substantia nigra.

LEARNING AND MEMORY: PHARMACOLOGY IV

410.1

GALANIN ANTAGONIZES ACETYLCHOLINE ON A MEMORY TASK IN BASAL FOREBRAIN-LESIONED RATS. J. Mastropaolo and J.N. Crawley. NSB , NIMH, Bethesda, MD 20892.

Galanin coexists with acetylcholine in nucleus basalis magnocellularis (NBM) and medial septal (MSA) neurons projecting to the cerebral cortex and hippocampus in primates (Melander and Staines, Neurosci Lett, 1986; Walker et al., Soc. Neurosci 1987), and in MSA neurons projecting to the ventral hippocampus in rats (Melander et al., Brain Res 1985), where galanin inhibits the release of acetylcholine (Fisone, PNAS, 1987). To investigate the possible behavioral role of galanin in memory processes thought to be mediated by these pathways, male Sprague-Dawley rats were lesioned with ibotenic acid at five sites in the NBM-MSA (Helpler et al., Brain Res 1985), and trained on a delayed alternation t-maze task for a food reward. Acetylcholine, 7.5 or 10 ug ivt, or 1 ug into the ventral hippocampus, significantly reversed the performance deficit in the lesioned rats. Galanin, 100-500 ng ivt, or 200 ng into the ventral hippocampus, attenuated the ability of acetylcholine, 10 ug, to restore t-maze performance in the lesioned rats. Galanin alone, 200 ng, partially reduced performance in the lesioned rats in the t-maze alternation task with no delay. At these doses, galanin had no effect on t-maze performance in sham-lesioned control rats. These data suggest that galanin may inhibit cholinergic function in brain pathways relevant to Alzheimer's disease.

410.2

BETA-ENDORPHIN ADMINISTRATION IMPAIRS AQUISITION IN THE CHICK. E.L. Bennett, T.A. Patterson, G. Schulteis, J.L. Martinez, Jr., & M.R. Rosenzweig. Department of Psychology, University of California, Berkeley, CA 94720

Several experiments, using a one-trial taste-avoidance task, were designed to examine the effects of beta-endorphin on memory formation in the chick. Previous results indicate that 5 min pre-training injection of beta-endorphin (0.01-1.0 nmole per hemisphere) into the medial hyperstriatum ventrale (MHV) produced amnesia after training (Patterson, et al., in press).

To determine the post-training susceptibility gradient for beta-endorphin, groups of chicks were given bilateral injections into the MHV of either saline or beta-endorphin (1.0 nmole per hemisphere), 5 min before training, or at various times after training. The chicks were tested 24 hr after training. The results indicate that beta-endorphin is amnestic when given 5 min before training, but is not amnestic when given at any time after training. These results suggest that this opioid acts on an early stage of memory formation.

To examine the time course of amnesia development, chicks were given bilateral injections into the MHV of either saline or beta-endorphin (1.0 nmole per hemisphere) 5 min before training, and groups of chicks were tested at various times after training. The results indicate that beta-endorphin produces amnesia that is present by 10 sec after training; this amnesia is permanent.

These results indicate that in chicks, as in rodents, pre-training injection of beta-endorphin produces an impairment in aquisition.

Supported by NSF grant BNS-86-06938 and NIDA grant 04195.

410.3

FURTHER STUDIES ON THE ROLE OF OPIOID DELTA RECEPTORS IN THE EFFECTS OF [LEU]ENKEPHALIN ON ACTIVE AVOIDANCE CONDITIONING IN MICE. G. Schulteis & J.L. Martinez, Jr. Dept. of Psychology, University of California, Berkeley, CA 94720

We previously reported (Neurosci Abstr 13:845, 1987) that selective stimulation of opioid delta receptors with [D-Pen^2-D-Pen^5]enkephalin (DPDPE) impairs acquisition of active avoidance, as does [Leu]enkephalin (LE), and that selective blockade of these receptors with ICI 154,129 enhances acquisition. Here we determine whether the effects of LE are reversed by ICI 174,864, a delta selective antagonist, and whether DAGO (Tyr-D-Ala-Gly-NMe-Phe-Gly-ol), a mu-selective agonist, affects conditioning. Peptide effects on locomotor activity were also assessed.

Two minutes prior to training (see Neurosci Abstr 13:845, 1987), male Swiss-Webster mice received ip injections of saline, LE, ICI 174,864, DAGO, or a combination of LE and ICI 174,864. LE (30 & 100 µg/kg) significantly impaired acquisition as measured by total avoidances over 14 trials. ICI 174,864 (3 mg/kg) significantly enhanced acquisition, as did DAGO (0.92 µg/kg). The dose response curves for all peptides were U-shaped. Finally, ICI 174,864 (1 mg/kg) reversed the impairment produced by LE.

Neither avoidance-impairing doses of LE (30-100 µg/kg), nor enhancing (3 mg/kg) or reversing (1 mg/kg) doses of ICI 174,864 had any effect on shock-induced activity levels measured in the avoidance apparatus. However, DAGO (0.92 µg/kg) increased activity levels; the enhancing effect of this peptide might thus be a performance effect. These data suggest that effect of LE on acquisition of active avoidance involves delta receptor activation. (Supported by PHS grant DA #04195 [JLM] and PHS NRSA #1 F31 DA05334-01 [GS])

410.4

[LEU]ENKEPHALIN AND ITS DELTA-SELECTIVE ANALOG, D-PEN^2-[D-PEN^5]ENKEPHALIN, IMPAIR AVOIDANCE CONDITIONING IN AN AUTOMATED SHELF-JUMP TASK IN RATS. S.B. Weinberger, C. Gehrig*, and J.L. Martinez, Jr. Psych. Dept., Univ. Calif., Berkeley, CA 94720, and Chem. Dept., Univ. Ariz., Tucson, AZ 85721

We reported previously that [leu]enkephalin (LE) impairs acquisition of a one-way active avoidance response in rats and mice, and that D-pen^2-[D-pen^5]enkephalin (DPDPE), a delta opioid receptor-selective analog of LE, also impairs acquisition in mice (c.f. Behav. Neural Biol. 49:192, 1988, for review). In the present study we extend these findings to an automated shelf-jump task in rats.

Male Sprague-Dawley rats received LE, DPDPE, or saline (ip) 5 m before placement in the apparatus. After 10 s a 330 uA shock was delivered to the grid floor. Rats avoided or escaped the shock by jumping to an elevated platform, where they remained for 30 s. They were then mechanically pushed off the platform to start the next trial.

LE (1 and 3 ug/kg) impaired acquisition as measured by total avoidances over 8 trials. DPDPE impaired performance at a dose equimolar to the 1 ug/kg LE dose, but not at doses equimolar to 0.1 or 10 ug/kg of LE. U-shaped dose-response curves also are seen in mice given LE and DPDPE. These data support our suggestion of delta opioid receptor involvement in LE's effects on acquisition.

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410.5

QUATERNARY FORMS OF NALOXONE AND NALTREXONE ENHANCE ACQUISITION OF ONE-WAY ACTIVE AVOIDANCE CONDITIONING IN MICE. E.A. Mendieta & J.L. Martinez, Jr. Dept. of Psych., Univ. Calif., Berkeley, CA 94720.

Pre-training ip injections of naloxonium (NXM) enhance acquisition of one-way active avoidance conditioning (Psychopharm., 87:410, 1985). However, naltrexone methyl bromide (NMB) failed to alter passive avoidance retention when administered post-training (Behav. Neural Biol., 44: 434, 1985). This apparent discrepancy may reflect: 1) differences in time of administration; 2) differences in conditioning paradigm employed; or 3) differences in pharmacological properties of NXM and NMB. The present study therefore compared the effects of pre-training injections of NXM and NMB on one-way active avoidance acquisition.

Male Swiss Webster mice were given [leu]enkephalin, NXM, NMB, or saline 2 minutes prior to training (see Neurosci. Abstr., 13:845, 1987). [Leu]enkephalin (100 ug/kg) impaired acquisition of the avoidance response. NXM (0.1, 1.0, & 10.0 mg/kg) enhanced acquisition. NMB (0.1 & 1.0 mg/kg) also enhanced acquisition, but 10 mg/kg was ineffective. These results suggest that both quaternary opioid antagonists exert similar modulatory effects on one-way active avoidance conditioning when administered prior to training. The effects of post-training injections of NXM and NMB on retention of active avoidance conditioning are currently being investigated. (Supported by PHS grant DA #04195).

410.6

EFFECTS OF CAPTOPRIL AND NALOXONE ON SHUTTLE AVOIDANCE EXTINCTION: EVIDENCE FOR OPIATE MEDIATION. A.Sudilovsky, B.A. Turnbull and L.H. Miller*. The Squibb Inst. for Med. Res., Princeton, NJ 08543 and Dept. of Biobehav. Sci., Boston Univ. Med. Ctr., Boston, MA 02118.

Earlier experiments (A. Sudilovsky et al, Abst. CINP, 397, 1984) indicated that captopril (C) delays extinction of shuttle avoidance response in the rat. In the current study, 48 male Sprague Dawley rats, 180 days old, were tested using a standard shuttle box and a 10-sec. tone as conditioned stimulus followed by the delivery of a 0.8 mA current to the chamber floor. Animals reaching at least 85% correct responses on the last 4 of 15 days of training (20 trials/day on a 30-sec. VI schedule) were randomly assigned (n=8/group) to C (10mg/kg), naloxone HCl (N, 0.25mg/kg), morphine sulfate (M, 5mg/kg), C plus N (CN), M plus N (MN) or saline (S). All solutions (1mg/ml) were given blind, i.p., on the 2 days prior to the 14-day testing period and also 1 hour prior to each daily testing (20 trials/day with no shock upon failure to shuttle) No significant differences were found post-hoc between the S and the N, M, CN, and MN groups. In contrast, C produced higher avoidance responding than N and CN (days 8-14, $p<0.01$), S and MN (days 9-14, $p<0.01$), and M (days 12-14, $p<0.01$). The finding that C-induced delay of shuttle avoidance extinction is suppressed by N indicates that the positive effects of C in this memory paradigm are, at least in part, mediated by endogenous opiates.

410.7

EFFECTS OF POSTNATAL TESTOSTERONE PROPRIONATE ADMINISTRATION ON RADIAL ARM MAZE PERFORMANCE IN RATS. R. L. Roof. Dept. of Psychology and Neuroscience Program, University of Wyoming, Laramie, WY., 82071.

This study examined the hypothesis that sex differences in spatial ability are due in part to differential steroid hormone environments during brain development. Male and female rats were injected with either 75 ug or 150 ug testosterone proprionate (TP) or an oil vehicle on days 4 and 6 after birth. At 90+ days of age, the rats were trained to a criterion level of performance on a radial arm maze. The rats were tested once a day for 10 successive days. Rats were then tested for 24 trials on a task in which only 4 of the 8 arms were baited. Accuracy and pattern of arms entered were recorded. Female rats treated with TP reached the criterion level of performance in significantly fewer trials than did control females, while the opposite pattern was observed for males. A dose related effect was observed for females, with the higher dose producing better performance. Control rats entered significantly more adjacent arms in sequence than did rats treated with TP. Males entered fewer adjacent arms in sequence than did females. No overall sex differences for accuracy were observed once criterion levels of performance were reached. These findings support the hypothesis that steroid hormones have an organizational effect on the developing brain that subsequently influences cognitive functioning.

410.8

CHRONIC TREATMENT WITH MORPHINE OR NALTREXONE INVERSELY AFFECTS RADIAL ARM MAZE PERFORMANCE OF RATS J.W.Spain* and G.C.Newsom* (SPON: H.L.Komiskey), Dept. Biomed. Sci., Univ. Ill. Col. of Med., Rockford, IL 61107

Several reports implicate the opioid system in learning and memory processes. To further explore this relationship, we utilized an appetitive task, the radial arm maze. Moderately food-restricted rats were chronically treated with either saline, morphine (40 mg/kg) or naltrexone (5 mg/kg) i.p. twice daily (two hours prior to testing and subsequent), begun 2 days before testing. Subjective behavioral effects of treatment were not obvious during testing. After the first week of testing, the protocol changed from allowing 8 choices to allowing retrieval of 4 rewards, removal for a two min delay, then return to the maze to try for the remaining four. Score one error for each duplication of an arm during a trial. With the delay, performance of all groups fell equally to approx. 1.5 errors. One week later, performance of naltrexone treated rats had significantly improved to only 0.6 errors, not to improve further in the subsequent 5 weeks. The performance of saline treated rats improved during the first two weeks following the start of the delay but, overall, did not achieve that of naltrexone treated rats. The performance of morphine treated rats did not improve during the 5 week course of treatment. When drug treatment was discontinued, there was no change for naltrexone or saline groups, but performance of morphine treated rats improved significantly in two weeks to equal that of saline-treated.

410.9

CHOLECYSTOKININ-OCTAPEPTIDE ENHANCES LEARNING AND MEMORY IN MICE AND MONKEYS. N. Pietrusiak*, L. Rumennik*, G. P. Vincent and J. Sepinwall (SPON:A. Davidson). Neurobiology and Obesity Research, Hoffmann-La Roche Inc., Nutley, NJ 07110.

CCK-8 has been reported to alter the acquisition or retention of learned responses in animals although the nature of its effect, improvement or impairment, has varied from study to study. In the present study, sulfated CCK-8 was evaluated in 2 species to assess its effects on learning and memory. CCK-8 (1-10 mg/kg s.c.) protected CF1 mice against electrobrain shock (EBS) disruption in retrieval of an active avoidance response; CCK-8 exhibited an inverted U-shaped dose-response curve. In C57BL/10 mice, CCK-8 (0.03-0.3 mg/kg s.c.) also significantly decreased the latency to find the hidden platform in a Morris water maze; the duration of action was 30 min. In squirrel monkeys, CCK-8 improved retention on a food-rewarded delayed match-to-sample procedure by 5-11% at doses of 0.0001-0.01 mg/kg i.m. These results suggest that CCK-8, administered parenterally, may have a modulatory influence on cognitive function, although the mechanism by which this occurs remains to be identified. Furthermore, compared to the usual appetite suppressant doses, rather high doses of CCK-8 were needed to enhance cognitive performance in mice whereas low doses were efficacious in the monkey.

410.10

EFFECTS OF DELTA-9-THC ON SIGNAL DETECTION AND DELAYED MATCHING TO SAMPLE PERFORMANCE IN RATS. C.J. Heyser*, C.R. Breese*, R.E. Hampson and S.A. Deadwyler, Dept. of Phys. & Pharm., Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC 27103

Previous reports from this laboratory showed that delta-9-tetrahydrocannabinol (THC), the psychoactive ingredient in marihuana, produced a dose-dependent disruption of tone discrimination behavior correlated with alteration of identified neural responses from the hippocampus (Campbell *et al.*, JPET 239 1986a,b). In the present study, two different behavioral conditioning tasks, delayed-matching-to-sample (DMTS) and tone detection (TD) were examined after injection of THC at previously utilized dose levels (0.5-2.0 mg/kg). In the TD task, animals were trained to respond to single tones (duration 250 msec) presented at 7 different intensities with the middle intensity set at behavioral detection threshold. Doses of THC above 0.5 mg/kg produced a dose-dependent decline in the detection of tones presented at or above threshold level ($p<0.0001$). Recordings of tone evoked potentials in hippocampus revealed differences between detect and non-detect trials. Cannabidiol, a nonpsychoactive derivative of marihuana, had no effect on these measures. In the DMTS task animals were required to perform a spatial discrimination following a 1-30 sec retention interval by selecting one of two levers (left or right) previously pressed. There was a marked dose-dependent disruption in performance ($p<0.0002$) by THC which was correlated with the length of the delay interval ($p<0.0001$). No significant disruption of performance occurred at the 1-5 sec intervals at any dose of THC. It is likely that THC had a selective effect on retention as manifested by influences on concomitantly recorded hippocampal neural activity. In comparison with previous reports (see above), the two tasks dissociate the effects of THC on detection of sensory events vs memory for trial specific information. Supported by NIDA Grants DA04441, DA03502, and DA00119 to S.A.D.

410.11

VASOPRESSIN ATTENUATES SCOPOLAMINE-INDUCED MEMORY IMPAIRMENTS IN RHESUS MONKEYS. M. Gravelle*, R.Q. Wan*, and T.G. Aigner (SPON: R. Brown). Lab of Neuropsychology, NIMH, Bethesda, MD 20892.

Arginine vasopressin (AVP) is an endogenous neuropeptide that has been reported to show reduced activity in Alzheimer's dementia (AD), a disease characterized by loss of cholinergic function and memory. AVP facilitates some retention processes in animals and humans, but attempts to reverse the memory loss of AD with analogs of AVP have been only partially successful. Because little is known about the interactions of AVP with the cholinergic system, we administered AVP alone and in combination with the anticholinergic agent scopolamine (SCOP) to 3 monkeys performing an automated memory test for food reward. The task required the animal to remember lists of graphic symbols (up to 9) that were projected onto a color monitor fitted with a touch-sensitive screen. AVP alone (1.0, 1.78, 3.2, or 5.6 ug/kg, s.c. 1 hr before test) had no effect, whereas SCOP alone (17.8 ug/kg, i.m. 20 min before test) produced a significant memory loss. When both compounds were given, AVP (1.78 and 3.2 ug/kg) significantly attenuated the SCOP-induced memory impairment, even though scores did not return to control levels. Although the mechanism for this attenuation is unknown, the results encourage further study of the possible therapeutic effects of AVP.

410.12

AVP 4-9 MODULATES MEMORY RETRIEVAL IN THE RAT AS A FUNCTION OF RETENTION INTERVAL. M.D. Bunsey* and B.J. Strupp. Dept. of Psychol., Cornell, Ithaca, N.Y. 14850.

Two recent studies in this lab have suggested that the effect of AVP4-9 on memory retrieval depends on memory strength. One study used a retention interval that produced little forgetting in controls and demonstrated impairment of retrieval with AVP4-9. In a second study, using a longer retention interval at which controls showed poor memory, pre-test AVP4-9 enhanced performance. This interaction must be viewed as tentative, however, as the AVP4-9 effects were obtained with different doses.

The present study directly examined this putative interaction using the same behavioral paradigm. It is known that if a rat is given a choice between two novel foods it will demonstrate a preference for the food that its cagemate had consumed prior to their interaction. In this experiment, rats were injected with AVP4-9 or vehicle and given a food choice after varying retention intervals (delay between cagemate interaction and food choice test).

Analysis of food preference at retention test revealed an interaction between drug condition and retention interval. AVP4-9 impaired recall at the retention interval at which memory was strongest in controls and enhanced recall at the interval at which memory was weakest in controls. These results support the hypothesis that AVP4-9's memory retrieval effects vary with memory accessability.

EPILEPSY: PEPTIDES

411.1

DIFFERENTIAL CHANGES OF THE LEVELS OF PROENKEPHALIN AND PREPRODYNORPHIN mRNAs IN RAT BRAIN DURING THE DEVELOPMENT OF DEEP PREPYRIFORM CORTEX KINDLING. P. H. K. Lee, C. W. Xie*, D. Zhao*, and J. S. Hong. Lab. of Molecular and Integrative Neuroscience, NIEHS, Research Triangle Park, NC 27709.

We have demonstrated by repeated subconvulsive electrical stimulations of the deep prepyriform cortex (DPC) led to an early increase of Met^5-enkephalin but not dynorphin in the hippocampus before the development of generalized convulsions. This study investigates whether such change is due to an increase in proenkephalin (pEK) mRNA during the development of DPC kindling. Rats were stimulated every hour until twice consecutive Stage 2 (S2) or Stage 5 (S5) occurred and were sacrificed either 5 min or 24 h later. At S2-24 h pEK mRNA in the entorhinal cortex and striatum was significantly increased. pEK mRNA was also increased in the hippocampus, frontal cortex, and entorhinal cortex 5 min after S5, whereas 24 h after S5, significant increases were found in the frontal cortex and entorhinal cortex but not in the hippocampus. Hippocampal preprodynorphin (pDYN) mRNA was significantly reduced 24 h after S5 but not at the other time periods studied. These data demonstrate that pEK and pDYN gene expressions were differentially regulated during the development of DPC kindling. The early increase in pEK mRNA content suggests that enkephalinergic system may play a role in kindling development.

411.2

ELECTROCONVULSIVE SHOCK ALTERS CONTENT OF mRNA CODING FOR PREPRODYNORPHIN AND PROENKEPHALIN IN DIFFERENT RAT BRAIN REGIONS. C. W. Xie*, P. H. K. Lee, J. Douglass[a], and J. S. Hong. Lab. of Molecular and Integrative Neuroscience, NIEHS, RTP, NC 27709 and Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR[a].

The effects of single or repeated electroconvulsive shocks (ECS) on abundance of dynorphin and enkephalin mRNA in rat brain were investigated. Rats were subjected to 1, 3 or 6 daily ECS and sacrificed 24 h later. The amount of mRNA coding for preprodynorphin (DYN mRNA) and proenkephalin (EK mRNA) were measured in several brain regions using RNA blot analysis. Repeated ECS led to a progressive decrease in hippocampal DYN mRNA with maximum reduction of 51% after 6 consecutive ECS. A consistent but lesser decrease in DYN mRNA level was observed in the entorhinal cortex 24 h after 1, 3, or 6 daily ECS. In contrast, DYN mRNA level showed significant elevation (by 83%) in striatum, and a modest increase (by 33%) in hypothalamus after a single ECS, which gradually declined after 3 or 6 daily shocks. EK mRNA was found to increase in entorhinal cortex (57%) and hypothalamus (75%), whereas only a small decrease was observed in hippocampus and no change in striatum after 6 daily ECS. These data suggest that repeated ECS enhances the biosynthesis of enkephalin but inhibits that of dynorphin in the entorhinal cortex-hippocampal region.

411.3

LOCALIZATION AND QUANTIFICATION OF VIP-, SS-, AND GABA-LIKE IMMUNOREACTIVITY IN THE HIPPOCAMPUS AND AREA DENTATA OF El (EPILEPTIC) AND C57BL/6 MICE. J.J. King, Jr.*, and C.G. LaMotte. (SPON:S.Kapadia) Section of Neurosurgery, Yale Univ. Sch. Med., New Haven, CT 06510.

The El mouse, an excellent genetic model of temporal lobe epilepsy, experiences tonic-clonic seizures of hippocampal origin following rhythmic vestibular stimulation. No previous work has documented an anatomical substrate for the seizure disorder. El mice were kindled by gentle tossing at age 30, 37 and 44 days and then tested for seizures after 56 days. At sacrifice all El mice had experienced at least 10 documented seizures. Presence of VIP, SS and GABA was determined using PAP immunohistochemistry. We observed elevated VIP-LI and GABA-LI interneuron cell densities in specific hippocampal anatomical subdivisions of El mice when compared to C57BL/6 mice. Increases of VIP-LI cell densities were seen in the stratum oriens and the regio superiore stratum radiatum. GABA-LI cell densities were greater in the stratum radiatum. There was no difference in the SS-LI cell densities. Both the VIP-LI and GABA-LI changes represent alterations in either the numbers of regulatory interneurons or their levels of transmitter synthesis. These data suggest that transmitter perturbations in the cells that modify projection cell output may be indicative of altered neural circuitry related to the epileptic predisposition of El mice.

The present study found differences in relatively "young" adult El mice without an extensive documented seizure history. The development of epilepsy in immature mice and the cumulative effects of many seizures in older animals will be examined in future studies. Additional immunohistochemical work on immature and aged animals may yield further insights into the epileptic process in the El mouse.

411.4

THE TIME COURSE OF CHEMICAL CHANGES IN THE KINDLED RAT BRAIN. N.S.Nadi. NINCDS, Bethesda, Md.

The evolution of changes in amino acids, neuropeptides and catecholamines were studies in the cortex (CX) and hippocampus (H) of kindled rats at stages i,ii,iii,iv and v of seizures. The levels of the amino acids, glutamate, aspartate, GABA and glycine were unchanged throughout the evolution of seizures in CX and H. Somatostatin increased at stage ii in CX but not H and continued to increase reaching a maximum at stage v in the CX. The changes in somatostatin in the H were not as marked as those observed in the cortex. The levels of substance P, neuropeptide Y and dynorphin were unchanged. The levels of enkephalin increased in the H at stages iv and v, but were unchanged at the earlier seizure stages. Enkephaloin levels were not markedly altered in the CX. The levels of catecholamines were not altered at any of the stages in CX or H. The number of NMDA receptors was slightly increased at stage ii in the H and was significantly higher compared to sham operated animals at stages iv and v in both H and CX.At stage iv and v the release of glutamate, aspartate and norepinephrine were significantly increased in the H and CX.The dynamic interactions between these changes are currently being investigated using slice techniques.

411.5

LAMINAR DISTRIBUTION OF PEPTIDES, CHOLINE ACETYLTRANSFERASE AND RECEPTORS IN THE HUMAN EPILEPTIC CORTEX. H.H.Drucker*, A.R.Wyler* and N.S.Nadi., NINCDS Bethesda, Md., and U.Tennn Med. Ctr. Memphis, Tn. (SPON: E.Streicher).

We measured the laminar distribution of choline acetyltransferase (CAT), neuropeptide Y(NPY), somatostatin(ST), glutamate (glu), aspartate (asp) and QNB and NMDA receptors in the temporal cortex (CX) removed from 8 individuals with intractable epilepsy. The layers were defined as follows : layer A: meninges, layer B: gray matter 1 and 2, layer C: gray matter 3 and 4, layer D: gray matter 5 and 6, and white matter. Glu was elevated in layer D of spiking (S) 15.8±2.8 umol/mg prot. vs nonspiking (NS) CX 8.1±3.4 umol/mg prot.. NMDA receptors were elevated in layer D 22.8 fmol/mg prot. of S vs NS CX 12.8±2.9fmol/mg prot.. CAT increased in layer B of S(35.4±6.8 pmol/mgprot/hr vs NS CX (20.7±11.9pmol/mgprot/hr). The enzyme was also elevated in layer C. ST was elevated in layer C in S (310±200 pg/mg prot) vs NS (160±150 pg/mg prot.) CX. NPY was increased in layer B of S (565±85 pg/mg prot) vs NS (345±145 pg/mg prot.) CX. No significant gradients in the distribution of asp and QNB were observed in the layers analyzed. The different distributions of the NPY and ST alterations suggest the involvement of two different populations of neurons. The changes in glu and NMDA receptors occuring in layer D indicate the involvement of yet another group of fibers and neurons in the excitatory amino acid pathways. The relationship of these findings to seizure generation will be discussed.

411.6

CONTENT OF NEUROTENSIN, VASOACTIVE INTESTINAL POLYPEPTIDE AND CHOLECYSTOKININ IN THE HUMAN EPILEPTIC CORTEX AND THE HIPPOCAMPUS. K.Wayns*, A.R.Wyler* and N.S.Nadi. NINCDS, Bethesda, MD., U.Tenn. Med. Ctr. Memphis Tn. (SPON: E.T.Hambrecht).

The levels of the neuropeptides neurotensin (NT), vasoactive intestinal polypeptide (VIP) and cholecystokinin (CCK) were determined in the spiking and non spiking regions of the temporal lobes surgically removed from 30 intractable epileptics. CCK was lower in the spiking cortex (12.5±2.8 pg/mg prot.) vs the non spiking cortex 22.9±1.7 pg/mg prot. ,p<0.01). NT was higher in the spiking cortex when compared to the non spiking cortex, 0.82±0.2 pg/mg prot. and 0.59±0.2 pg/mg prot. respectively (p<0.01). VIP was also lower in the spiking vs non spiking cortex, 31.4±12.1 pg/mg prot. vs 62.4±10.4 pg/mg protein respectively, (p<0.01). The levels of NT in six epileptic hippocampi were 0.79±0.03 pg /mg prot.. The levels of VIP and CCK were 25.5±0.82 and 11.9±0.92 pg/mg prot. respectively. No autopsied hippocampus was available for comparison. The role for most neuropeptides in the central nervous system is unclear. NT is known to colocalize with dopamine (DA) neurons and also to be excitatory. Our previous studies have shown an increase in DA in the spiking cortex and it is possible that NT and DA may be colocalized. NT may also regulate the excitability of the region. VIP has been implicated in control of circulation and the alterations observed suggest a disorder in regional circulation.

411.7

SECOND MESSENGER SYSTEMS IN THE KINDLED CORTEX AND HIPPOCAMPUS. N. Mai* and N.S.Nadi. NINCDS, Bethesda, Md. (SPON: W.H.Theodore).

We have investigated the effects of norepinephrine (NE), increased K+, N methyl aspartate (NMA) with respect to cyclic AMP and cyclic GMP regulation in slices prepared from the cortex and the hippocampus of the stage v kindled rat. Increased K+ caused a significantly larger increase in the kindled rat hippocampus and cortex slices than in the slices from sham operated rats. The changes in cyclic AMP content were not statistically different between kindled and sham operated rat brain slices. The levels of cyclic AMP were increased in response to exposure of the cortex and the hippocampus slices to norepinephrine. These changes were not statistically different between the kindled and sham operated animals. NMA cuased a significant elevation in cyclic GMP in both the cortex and hippocampus slices in the kindled and sham operated rats. The increase in cyclic GMP in the kindled cortex and hippocampus slices was significantly higher when compared to the sham operated hippocampus and cortex. These preliminary experiments suggest that the control of the cyclic GMP pool is altered in the cortex and the hippocampus of the kindled rat brain. Such alterations in second messenger control may represent a step in explaining the molecular mechanisms of the changes underlying the kindling phenomenon. Studies are currently underway to determine the time course of these changes as well as the effect of the above compounds on the phosphotidylinositol class of second messengers.

411.8

A CHEMICAL EVALUATION OF THE EPILEPTIC FOCUS. A.R.Wyler*, N.S.Nadi. U. Tenn. Med.Ctr. Memphis Tn., and NINCDS, Bethesda Md. (SPON: W.W.Alberts).

Previous studies in our laboratory have discovered a number of alterations in neuropeptides, catecholamines amino acids, receptors and enzymes in the human epileptic focus. With results from 35 patients we have analyzed the data with respect to cross correlations between these findings as well as lateralization, correlations with anticonvulsant drug levels, male-female and age related differences and relationship to surgical outcome. The question addressed in these analyses was the usefulness of the biochemical studies of the spiking brain and what future approaches might be inferred from biochemical studies. The cross correlations of all the data revealed a statistically significant correlation between somatostatin and norepinephrine (r=0.82, p<0.001), and ST and dopamine (DA) (r=0.62, p<0.01). Neurotensin (NT) and DA were also significantly correlated (r=0.52, p<0.01). The transmitter metabolites HVA and 5HIAA were also correlated (r=0.79, p<0.01). No other statistically significant correlations were demonstrated in this study. There was no significant lateralization of the transmitters although the catecholamines tended to be slightly lower in the left temporal lobe. No significant male- female or age related differences were noted. With respect to anticonvulsant drugs the levels of ST in patients on carbamazepine alone was slightly less than in patients on phenytojn alone. The data reported here did not correlate with surgical outcome. The strong correlations between ST, NT, DA and NE is suggestive of colocalization or transynaptic regulation. Such changes in the epileptic focus are suggestive of an altered synaptic connectivity as a result of neuronal loss. The lack of correlation with outcome may reflect the fact that surgery only removes a portion of the synaptic circuitry necessary to interrupt the seizures, but that it is not always necessary to remove all of the tissue to have good outcome. The data will be discussed with respect to animal models and a chemical identification of the spiking region.

411.9

INDOLES, CATECHOLS AND TYROSINE HYDROXYLASE IN THE HUMAN EPILEPTIC CORTEX. M. Pintor*, S. Pocotte, I. Mefford*, A.R. Wyler* and N. S. Nadi (SPON: R.J.Porter) . NINCDS, Bethesda, Md, and U. Tenn. Med. Ctr., Memphis, Tn.

The levels of serotonin (5HT), dopamine (DA), and their metabolites, 5HIAA and HVA were measured in surgically resected human spiking and nonspiking temporal cortex. In 13 pairs of spiking and non spiking cortex evaluated as pmol/mg protein 5HT was 6.1±2.4 and 3.5±1.3 respectively (p<0.01), and 5HIAA was 13.8±5.2 and 7.9±5 respectively (p<0.02). In a previous study norepinephrine (NE) was found to be 1.5±0.6 in the spiking cortex and 1.06±0.4 in the nonspiking cortex(p<0.001) and dopamine was 1.0±0.5 and 0.7±0.3 (p<0.001) (Goldstein, D.S, J.Neurochem. 50:225, 1988). No difference in the levels of the indoles and the catechols were observed between the right and the left temporal lobes. The activity of tyrosine hydroxylase (TH) was also measured and no difference was detected between spiking and non spiking regions of the temporal lobe. These results do not support previously reported findings (Sherwin, A. L., Neurology, 34:927, 1984). One reason for this discrepancy may be in the observation that the TH is increased during and immediately after seizure but not in the interictal phase as shown in the case of rats given a single electroconvulsive shock. The chronically elevated content of indoles and catechols may be due to an increased turnover and altered release rate. The existence of such changes in the human brain and kindled rats is currently being investigated using in vitro slice techniques.

411.10

HIPPOCAMPAL VOLUMETRIC NEURONAL DENSITY IN TEMPORAL LOBE EPILEPSY. J. H. Kim, P.O. Guimaraes*, M.Y. Shen*, C. Deutch*, S.S. Spencer, D.D. Spencer. Dept's. of Surgery (neuropathology, neurosurgery) and Neurology, Yale Univ. School of Medicine, New Haven, CT 06510

Hippocampal sclerosis, shown in a majority of temporal lobe epilepsy cases, is histologically composed of neuronal loss and gliosis, and its exact pathogenesis is controversial. To study the relationship between the degree of neuronal loss and clinical variables, volumetric neuronal density was measured in the hippocampal fields from the mid or mid-posterior level of surgically removed hippocampal coronal sections from 24 intractable epilepsy cases. 11 age matched autopsy cases without any previous history of seizure or other neurologically significant diseases served as control. Cell counts were done in multiple unit areas on 6um thick, paraffin embedded, hematoxylin and eosin stained sections in the CA1 through CA4 and granular layer. The counts were modified with Abercrombie formula, and were expressed as mean neuronal number per mm3. Pearson's correlation showed moderate to high correlation between the age of patients at the onset of seizures and the neuronal density in CA1 and CA2 (r=0.678, p<0.001, and r=0.777, p<0.001, respectively). A similar degree of correlation was obtained between a history of febrile seizures and CA1 neuronal density (r=0.666, p<0.001). No significant correlation was seen between the cell count and other clinical variables including age at the time of hippocampectomy, sex, duration of seizures (year), seizure frequency per month, frequency of seizures per month x duration in years, family history of epilepsy, and history of other neurologic problems such as head trauma or CNS infection. Thus, early onset of seizures and a history of febrile seizures are associated with statistically significant loss of neurons in CA1 and CA2, and in CA1, respectively.

411.11

Anatomic - Pharmacologic Analysis of Benzodiazepine Receptor Binding in Human Epileptic Hippocampus. H.T. Chugani*, T.L. Babb, W.R. Kupfer* and J.K. Pretorius* (SPON: E. Rubinstein). Depts of Neurology and Pediatrics and the Brain Research Institute. UCLA School of Medicine, Los Angeles, CA. 90024.

Benzodiazepine receptor binding was measured using [^{3}H]-flunitrazepam quantitative autoradiography of the fascia dentata of 6 patients who underwent temporal lobectomy for relief of epilepsy. Quantitative estimates by Scatchard analysis of receptor densities (Bmax) and affinity constants (K_D) were compared to granule cell counts of the same region in order to relate cell loss to cell receptor binding properties when using an autoradiographic densitometric technique. Mean Bmax values were 213.5 ± 43.1 SEM and mean K_D values were 5.2 nM ± 1.5 SEM. However, Bmax values ranged from 160.0 to 425.5 fmol/mg tissue and correlated inversely with the percentage of granule cell loss (R=0.54). K_D was not correlated with granule cell loss. These results suggest that receptor density in epilepsy may be determined by cell loss alone, while K_D is unaffected by hippocampal sclerosis. However, the correlation with cell loss in these analyses were not statistically significant until the data were described by a third-order polynomial. The interpretation of ligand autoradiography using light transmission must take into account the underlying anatomy of the cell densities and dendritic processes that may be labelled. NIH Grant NS02808.

411.12

Altered Patterns of Dynorphin-like Immunoreactivity in the Hippocampal Formation of Patients with Temporal Lobe Epilepsy. C.R. Houser and J.E. Miyashiro*. VA Medical Center, West Los Angeles and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Dynorphin A^{1-17} has been localized in immunocytochemical preparations of human hippocampal formation. In control autopsy specimens, dynorphin A-like immunoreactivity was present in the mossy fiber path, as demonstrated in the rat (McGinty, J.F. et al., 1983), extending through the hilus and CA3 field. Very little reaction product was evident in the molecular layer of the dentate gyrus. In contrast, in surgical specimens from patients with temporal lobe epilepsy, dynorphin-like immunoreactivity was present in the granule cell and molecular layers, sometimes extending through the inner third of the molecular layer. Reaction product was also concentrated around the limited number of neurons remaining in the hilus and CA3 field.

These findings suggest that morphological reorganization of mossy fibers has occurred in humans with temporal lobe epilepsy, as demonstrated in animal models of seizure disorders (Nadler, J.V. et al., 1980), and indicate that the reorganized fibers contain at least one of the neuroactive substances normally present in mossy fibers. Supported by VA Medical Research Funds and NIH Grant NS 21908.

411.13

GLUCOCORTICOID ANTAGONISM WITH RU 486 ENHANCES LIDOCAINE KINDLING. M. A. Kling, M. A. Demitrack*, M. DeBellis*, S. R. B. Weiss*, G. P. Chrousos*, R. M. Post, and P. W. Gold*. BPB, NIMH and DEB, NICHD, Bethesda, MD 20892.

Local anesthetics such as lidocaine are capable of producing kindled seizures in rats; i.e., initially subconvulsive doses will produce seizures when given repeatedly. The mechanism of this effect has not been definitively established; however, it may involve activation of limbic substrates based on metabolic autoradiographic data. We were interested in the potential involvement of corticotropin-releasing hormone (CRH) in this effect, since CRH causes limbic seizures which cross-sensitize with amygdala-kindled seizures, and local anesthetics stimulate CRH secretion in vitro. We report here a study of the effects of RU 486, a potent glucocorticoid antagonist expected to increase endogenous CRH levels via blockade of glucocorticoid negative feedback, on lidocaine kindling in rats. Male Sprague-Dawley rats received daily injections of either RU 486 (100 mg/kg, i.p.) or vehicle 2 hours prior to lidocaine HCl (65 mg/kg x 8 days, then 75 mg/kg X 9 days, i.p.). RU 486 significantly enhanced the development and cumulative frequency of lidocaine-induced seizures (chi-sq=14.4, p<.001). RU 486-treated rats also had significantly more mortality than controls (38.0% vs. 5.3%, chi-sq=6.2, p<.025). In 62% of the RU-treated rats, the deaths occurred following seizures. The facilitation of lidocaine kindling by RU 486 may be mediated in part by increased secretion of CRH. Alternatively, glucocorticoids, which enhance GABA-mediated chloride flux under some conditions, may be tonically inhibitory to lidocaine kindling. The increased mortality in the RU 486-treated rats may be due to diminished capacity of these rats to tolerate stressful conditions.

411.14

EFFECT OF NALTREXONE ON CRH-INDUCED SEIZURES IN RATS. G.I. Perini*, S.R.B. Weiss*, M.A. Kling, M. DeBellis*, L. Wilson*, K. Vogelsong*, G.P. Chrousos*, R.M. Post, P.W. Gold* (Spon: J. Ferguson) BPB, NIMH & DEB, NICHD, Bethesda, MD 20892

Corticotropin-releasing hormone (CRH) is a stress-related peptide which causes seizures of late onset [1-5 hour latency following intracerebroventricular (i.c.v.) administration] that involve limbic structures. In vitro, CRH causes the release of B-endorphin, a peptide which produces kindled seizures following intra-amygdaloid or hippocampal administration (Cain & Corcoran, 1984). On the other hand, endogenous opiates released during seizures may be related to post-ictal decreases in seizure susceptibility. To evaluate the role of opiate peptides in CRH-induced seizures, we administered naltrexone, an opiate antagonist (10 mg/kg, i.p.) one-half hour prior to, and 4 hours after (5 mg/kg) CRH (100 µg, i.c.v.) on 2 consecutive days. The overall effect of naltrexone was to potentiate CRH-induced seizures. Although the number of animals experiencing seizures did not differ on day 1, the number of seizures per animal tended to be greater in the naltrexone group than in the controls ($\bar{x}$ ± S.E.M.=5.71 ± 1.52 vs 2.37 ± 0.53); the latency tended to be shorter in the naltrexone-treated rats (3/7 rats had seizures within the first hour compared to 0/8 controls). On day 2, more naltrexone-treated rats tended to exhibit seizures (6/9 compared to 3/10 controls), although the overall number of seizures on this day did not differ between groups. Thus, opiate release is unlikely to be the mechanism responsible for CRH-induced seizures. The trend for an overall proconvulsant effect of naltrexone on several measures may be related to a blockade of post-ictal opiate effects which in other seizure models are inhibitory.

411.15

EFFECTS OF ESTROGENS ON EPILEPTIFORM ACTIVITY IN THE IN VITRO HIPPOCAMPAL SLICE. D. L. Pettit*, S. Veregge, D., Doherty*, and C. Dolorfo*. Dept. of Biology, San Jose State Univ., San Jose, CA 95192.

Many investigators have reported cyclic changes in seizure frequency in women with epilepsy. Increases in seizures have been observed just before menses or at ovulation, times when estrogen levels are high relative to progesterone. These observations, paired with those from in vivo animal studies, suggest that estrogens may potentiate epileptiform activity. We used the hippocampal slice as a model for investigating the direct short-latency effects of estradiol and estrone-3-sulfate, an estradiol metabolite, on epileptiform activity.

Hippocampal slices were prepared from proestrus female rats and maintained under standard incubation conditions. Epileptiform activity was produced using penicillin (3000 u/ml) in ACSF. Orthodromically evoked epileptiform field potentials were recorded from CA1 and the parameters of the field potentials (peak amplitude, duration and number of population spikes) were quantified before and after the addition of estrogens to the ACSF.

Estradiol did not modify the amplitude, duration of the epileptiform activity, or number of population spikes in either physiological (0.36-36 nM) or pharmacological (367 µM) doses. Estrone-3-sulfate modulated penicillin induced epileptiform activity in pharmacological (45-4500 µM), but not physiological concentrations. In addition, concentrations 45 µM or greater of estrone-3-sulfate produced epleptiform activity. The effects of estrone did not appear to depend on estrous stage or sex. These results suggest that estradiol does not modify epileptiform activity in the hippocampus at either physiological or pharmacological doses. Investigations of the mechanism of action of estrone and other metabolites are currently underway. Work supported by the Epilepsy Foundation of America.

411.16

DIETHYLDITHIOCARBAMATE AND DITHIZONE AUGMENT THE TOXICITY OF KAINIC ACID. C. L. Mitchell, M. I. Barnes*, and L. Grimes. Lab. of Molecular and Integrative Neuroscience, NIEHS/NIH, Research Triangle Park, NC 27709 and Toxicology Curriculum, UNC-CH, Chapel Hill, NC 27514.

Grimes et al. (J. Neurosci., 8:256-264, 1988) reported that colchicine-induced lesions of the hippocampal mossy fiber pathway eliminate kainic acid (KA)-induced wet dog shakes (WDS) but do not affect the latency to onset of seizures. Since chelation of zinc appears to result in a functional disruption of this pathway (Crawford and Connor, Orthomolec. Psychiat. 4:39, 1975) we wished to determine the effects of the zinc chelators, diethyldithiocarbamate (DEDTC) and dithizone (D) on KA-induced WDS and seizure activity. Male Fischer-344 rats were injected i.p. with DEDTC (100, 200, or 400 mg/kg) or D (12.5, 25, 50, or 100 mg/kg) 15 min. after KA (8 mg/kg, s.c.). DEDTC and D reduced both the number of WDS and the onset of seizures induced by KA. Moreover, they increased the severity of seizures and frequency of death. Doses as low as 100 mg/kg of DEDTC and 12.5 mg/kg of D were effective. For example, 7/10 animals receiving DEDTC (100 mg/kg) exhibited hind limb clonic seizures and 8/10 died following KA whereas these effects were seen in only 2/10 of the animals receiving KA plus the vehicle control. These compounds may be useful tools for investigating the role of Zn in central nervous system excitatory neurotransmission and/or convulsive phenomena.

411.17

EFFECT OF PYRIDOXINE ON THE DEVELOPMENT OF AMYGDALOID KINDLED SEIZURES IN THE RAT. L. Pao, M.A. Bixler, J.L. Meyerhoff (SPON: L.H. Hicks). Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C., 20307-5100.

Several studies implicate pyridoxine-dependent inhibitory mechanisms in a variety of seizure-inducing paradigms. Dietary pyridoxine deficiency is reported to decrease seizure latency in rats following systemic kainic acid challenge (Ruth, R.E. and Morgan, D.G., Exp. Neurol., 94:441, 1986). Pyridoxine injections 1 hour prior to insulin treatment inhibited insulin-induced seizures (Saad, S. F., Eur. J. Pharm., 17:152, 1972). Pyridoxine also increased the threshold for seizures in previously fully-kindled cats (Shouse, M.N., Exp. Neurol., 75:79, 1982). In the present study, pyridoxine injections (50 mg/kg, i.p.) were given to male Sprague-Dawley rats 1 hour prior to daily amygdaloid kindling stimulation (200 microamperes, biphasic, base-to-peak) and continued daily until fully generalized (stage 5) convulsions were obtained. The number of stimulations required to achieve the first stage 5 convulsion was not significantly different in the pyridoxine group (10.1 ± 1.08) compared to saline-injected controls (11.7 ± 1.09), t = 1.04, N = 17. Thus, despite the inhibitory effect of pyridoxine on seizures in a number of acute paradigms, it did not appear to retard the kindling process.

411.18

ANTICONVULSANT CONCENTRATIONS IN CSF AND SERUM OF RATS PROTECTED AGAINST MES BY PR934-423A. T.C.M. Wilson*, G.E. Machulskis*, G.E. Garske* and E.W. Harris (SPON: A.J. Jacobs). Analytical Chemistry & Pharmacology Departments, Pharmaceutical Div., Pennwalt Corp., Rochester NY 14603

The hydrochloride salt of PR934-423 ((+)-2-amino-N(1-methyl-1,2-diphenylethyl) acetamide.HCl; PR934-423A) is a water-soluble anticonvulsant effective in mice and rats against maximal electroshock seizures (MES), with a larger therapeutic index than carbamazepine, phenobarbital or valproate. To facilitate clinical and in vitro studies, PR934-423 concentrations were determined in serum and cerebrospinal fluid (CSF) of rats protected against MES.

Young adult male rats were dosed orally with PR934-423A at 19.7 or 86 mg free-base/Kg. At 1 or 3 hours post-dose, serum and CSF samples were taken and anti-MES efficacy was determined in separate rats. The concentration of PR934-423 metabolites was measured using 14-C-labelled drug. The total amount of drug-derived material (radiolabel, in uM) was determined 1 hour post-dose.

Only 4/12 rats were protected against MES 1 hour after 19.7mpK PR934-423, but 11/11 were protected 1 hour after 86mpK; 18/27 were still protected 3 hours after 86mpK. The corresponding CSF concentrations were 31, 153, and 53 ng/ml (0.11, 0.54 and 0.19 uM). The studies with radiolabel showed that PR934-423 accounts for approximately half of all drug-derived material in CSF.

PR934-423, or a metabolite, is a very potent anticonvulsant effective at ~0.5uM or less in CSF.

411.19

DORSAL AND VENTRAL HIPPOCAMPAL KINDLING IN FEMALE RATS WITH AND WITHOUT ESTRADIOL. J.S. Kiefer*, G.M. Hudson* and G.G. Buterbaugh (SPON:P.Monroe). Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Ovariectomized female Sprague-Dawley rats were kindled by daily stimulation of the dorsal (DH) or ventral hippocampus (VH). DH rats with estradiol (E) replacement required fewer afterdischarge (AD) trials to kindle (28.3 ± 3.5) compared to rats without (nE) estradiol (42.5 ± 3.7); E rats also accumulated 35% less AD time during kindling. E altered both early and late phases of DH kindling. In E rats, two landmarks of kindling, increased AD duration and first stage 4 generalized convulsion, appeared more quickly compared to nE rats. After the first stage 4 response, E rats required substantially fewer AD trials and sec to complete kindling compared to nE rats. Estradiol also stabilized late kindling generalized seizures since E rats rarely regressed to lower seizure stages as was common in nE rats. In contrast to DH kindling, VH kindling does not appear to be sensitive to estradiol. The results add to the evidence that the interaction of E with kindled seizure acquisition is stimulation-site dependent and keyed to generalized seizures. E may interact within a limited spatial domain in concert with the recruitment of parallel kindling circuits. The results further support a complex role for estradiol in catamenial epilepsy. (Supported by NS20670)

411.20

EFFECTS ON CORTICAL NEURONS OF THE KAPPA AGONIST U-50488H, THE ANTICONVULSANT U-54494A AND RELATED NON-KAPPA 1,2 DIAMINES. M.Camacho-Ochoa, G.D.Vogelsang, P.F.VonVoigtlander, and M.F.Piercey. The Upjohn Company, Kalamazoo, MI 49001.

U-50488H, a selective agonist for opiate kappa receptors, U-54494A, a non-kappa agonist anticonvulsant, and U-63764 and U-63640, two 1,2-diamines chemically related to U-50488H and U-54494A, were tested for effects on spontaneous and glutamate evoked firing rates in cerebral cortex of urethane-anesthetized male Sprague-Dawley rats. Standard 7-barrelled microelectrodes were filled with 200 mM, pH 5.5 concentrations of 1,2-diamines (except U-63764, 150 mM), or 200 mM glutamate (pH 8.0), kainic acid (pH 8.0), procaine (pH 5.5), glutamate diethyl ester (GDEE, pH 4.0), and naloxone (pH 6.0). Iontophoretic application of 1,2-diamines, GDEE, or procaine depressed spontaneous and amino acid-induced firing of cortical neurons. With continued ejection of 1,2-diamines or procaine, firing was silenced completely, but GDEE could maintain a partial suppression. A rapid rebound of excitation followed cessation of procaine ejections, but not of other agents. Iontophoretic naloxone failed to antagonize U-50488H or other 1,2-diamines. U-54494A, in doses up to 30 mg/kg i.v., significantly depressed spontaneous firing rates in 2 of 6 cortical neurons, but only 1 of 6 hippocampal pyramidal cells were weakly depressed. It is concluded that U-50488H and other 1,2-diamines inhibit neuronal excitability by a non-kappa receptor mechanism.

EPILEPSY: GABA AND BENZODIAZEPINES

412.1

REGIONALLY SPECIFIC EFFECTS OF DIAZEPAM ON 2-DG UPTAKE DURING STATUS EPILEPTICUS IN KINDLED RATS. B.E. Jones, G.M. Hudson* and G.G. Buterbaugh. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Pilocarpine-facilitated status epilepticus (pfSE) results from a synergism between seizure activity and a nonconvulsive dose of pilocarpine (20 mg/kg) in kindled rats. The early time-course (i.e.,60 min) of pfSE is characterized by stable generalized EEG seizures with mild motor components. We examined 2-DG uptake patterns during early pfSE in rats treated after 10 min of pfSE with a dose of diazepam (12.5 mg/kg; i.p.) resulting in akinesia with no overt change in EEG seizures. ^{14}C labelled 2-DG (100 uCi/kg; i.v.) was administered 5 min later; rats were sacrificed 45 min later and brains rapidly removed and frozen sectioned for contact autoradiography. Rats receiving vehicle only showed extensive bilateral 2-DG uptake particularly in the lateral septum, hippocampus, amygdala, most thalamic nuclei, substantia nigra, medial geniculate and all cortical areas. Diazepam treated rats showed a similar uptake pattern except for a selective bilateral decrease of 2-DG uptake in the entire extent of the hippocampus and parietal neocortical areas. These results suggest that the hippocampus and some neocortical areas are not significant factors in the maintenance of seizure activity in pfSE. (Supported UMAB DRIF Grant)

412.2

EXCITATION BY GABA IN MOUSE SPINAL CORD.
G. Czéh* and G.G. Somjen,
Dept. Physiol. Duke Univ. Durham NC 27710.

Last year we reported that spontaneous activity is inducted in hemisected mouse spinal cords *in vitro* by elevated $[Ca^{2+}]_o$ (Neurosci. Soc. Abstr. 13:318, 1987). Similar activity occurs in low $[Mg^{2+}]_o$. High $[Ca^{2+}]_o$ and low $[Mg^{2+}]_o$-induced spontaneous discharges in both dorsal and ventral roots (DRs and VRs) were blocked by the GABA-antagonists picrotoxin and bicuculline. After cleaving dorsal from ventral cord quadrant, spontaneous activity ceased in VR but persisted in DR. A short latency DR reflex (DRR1) could be evoked in all spinal cords in control solution (1.2 mM $[Ca^{2+}]$ and $[Mg^{2+}]$). DRR1 and the segmental monosynaptic reflex (VRR) were depressed in low $[Ca^{2+}]_o$ and in high $[Mg^{2+}]_o$ and slightly increased in high $[Ca^{2+}]_o$ and in low $[Mg^{2+}]_o$. Varying $[Ca^{2+}]_o$ had much stronger effects on the slow dorsal root potential (DRP). A later DR reflex (DRR2) appeared in elevated $[Ca^{2+}]_o$ (threshold of 1.8 mM). DRP and DRR2, but not DRR1, were depressed by bicuculline. We conclude that high $[Ca^{2+}]_o$- and low $[Mg^{2+}]_o$-induced spontaneous activity depends on an obligatory GABA-ergic link operating in dorsal gray matter. This confirms an excitatory effect of GABA in spinal cord (see Duchen: Brain Res. 379:182, 1986).
(Supported by grants NS 17771, NS 18670 and NS 06233)

412.3

GABA-STIMULATED CHLORIDE FLUX IN THE KINDLING MODEL OF EPILEPSY. W.M. Burnham, S.J. Kish* and W.B. Sneddon*. Dept. of Pharmacology, University of Toronto, Toronto, Ont. and Clarke Institute of Psychiatry, Toronto, Ont.

On the basis of preliminary data, Burnham et al. (1988) have recently suggested that GABA-mediated chloride flux is reduced in the brain stems of entorhinal-kindled rats. The present full-scale study confirms this finding, and indicates that significant changes may be found at both 24 hours and 28+ days after the last kindled seizure.

GABA-mediated chloride flux was assayed in the brains of entorhinal-kindled and implanted control rats, sacrificed either 24 hours or 28+ days after the 6th stage-5 kindled seizure. The assay, adapted from Hollingsworth et al., involved measurement of 36-chloride entry into synaptoneurosomes in the presence or absence of 100uM GABA. When cerebral cortex and cerebellum were assayed, no differences between kindled and control brains were found. When the brain stem was assayed, however, GABA-stimulated chloride flux was found to be greatly reduced in kindled subjects. Similar reductions were seen both at 24 hours and 28 days.

These data suggest the presence of a long-lasting deficit in GABA function in at least one area of kindled brain. They are consistent with a "GABA hypothesis" of kindling.

412.4

ALLELIC DIFFERENCES IN $GABA_A$ RECEPTORS BETWEEN SEIZURE PRONE AND RESISTANT MICE. J.C.R. Fernando and D.R. Burt. Dept. Pharmacol., U. Md. Sch. Med., Baltimore, MD 21201.

Inbred strains of mice differ markedly in their susceptibility to audiogenic seizures: juvenile DBA/2 mice are much more sensitive than C57BL/6 mice of any age. Strain differences in structure or expression of inhibitory GABA receptors may contribute to seizure susceptibility. We have looked for restriction fragment length polymorphisms (RFLPs) which distinguish genes for $GABA_A$ receptors in DBA/2 and C57BL/6 mice. Southern blots of genomic DNA extracted from the livers and cut with a variety of restriction endonucleases were hybridized at low stringency with cDNA probes based on published bovine $GABA_A$ receptor sequences (Schofield et al., Nature 328:221, 1987). Two enzymes have yielded clear RFLPs. PstI-cut DNA, when probed with a cDNA representing the N-terminal portion of the alpha subunit, gave the following pattern of bands (approx. sizes in kbp): DBA/2: 1.10, 1.87, 4.9, 6.9, 10.0; C57BL/6: 1.06, 1.80, 4.9, 6.9, 10.0. HindIII-cut DNA, when probed with a cDNA representing the C-terminal portion of the alpha subunit, gave: DBA/2: 1.2, 1.9, 3.1, 5.7; C57BL/6: 1.2, 1.9, 5.7, 8.0. The first pattern suggests small deletion(s) while the second suggests single base change(s) as strain differences in genes coding for one or more forms of the mouse $GABA_A$ receptor. The nature of these allelic differences is currently under investigation.

412.5

PERIPHERAL-TYPE BENZODIAZEPINE LIGANDS REGULATE SPECIFIC ^{3}H-PHENYTOIN BINDING TO RAT BRAIN WHOLE CORTEX MITOCHONDRIA. **J. FRANCIS and L. SPERO, Dept. of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.**

These studies were conducted to assess specific binding sites for ^{3}H-phenytoin (^{3}H-DPH) in rat brain and the regulation of these sites in vitro. These sites, previously reported to be concentrated in the P2 fraction of rat brain homogenates, were localized in the mitochondrial fraction but not in synaptosomes. Peripheral-type benzodiazepines (P-BZD), whose receptors are found in mitochondrial outer membrane, modulated specific ^{3}H-DPH binding to mitochondrial fractions. P-BZD agonists enhanced both total and specific ^{3}H-DPH binding in the order Ro5-4864 diazepam flunitrazepam Ro5-5115. PK11195, a specific P-BZD receptor antagonist, did not significantly modulate specific ^{3}H-DPH binding except at high (10 uM) concentrations, where partial inhibition of ^{3}H-DPH binding occurred. Central-type benzodiazepine (C-BZD) agonists and antagonists also did not significantly modulate specific ^{3}H-DPH binding in mitochondria, except at high (uM) doses (clonazepam, a C-BZD agonist, partially inhibits). Similar results were found in rat cerebellum. There is an apparent association between specific ^{3}H-DPH binding sites and P-BZD binding sites in rat brain cortex mitochondria. The high (uM) P-BZD concentrations involved suggests that the P-BZD binding site involved differs from the high (nM) affinity mitochondrial P-BZD receptor.(Supported by an O.M.H.F. Studentship to J.F.)

412.6

HIPPOCAMPAL GABA AFTER SYSTEMIC KAINATE (KA). P.E. Ruth and A. Selmen*. Il. Inst. Dev. Disabil., and the Committee on Neurosci., Univ. Il., Chicago, IL 60608.

We observed an apparent potentiation of diazepam (DZP) action by KA. DZP (5 mg/kg, ip) 10 minutes prior to a high dose of KA (>19 mg/kg, ip) prevented motor seizures, but the drug interaction caused respiratory depression and death in over 50% of the rats. Also DZP (10--20 mg/kg) 30 minutes after a more typical dose of KA (12 mg/kg) caused animals to "sleep" longer than expected from DZP alone.

[GABA] is implicated in this latter phenomenon. Adult female rats injected with KA (12 mg/kg, ip) were killed 30 minutes later by cervical dislocation. Half of the carcasses (3 KA-treated, 3 control) immediately received 5 seconds of cerebral microwave irradiation: for the other half, irradiation was delayed 3 minutes to estimate post-mortem GAD "activation." Hippocampal [GABA] was measured by radioreceptor assay.

In KA-treated rats, [GABA] was 19% higher than the control level of 1.62 ± 0.26 µmol/g (wet). The rate of post-mortem GABA accumulation in KA-treated rats was 27% lower than the control rate of 0.22 µmol/g/min. Paradoxically, [GABA] is elevated by KA at a time when postsynaptic inhibition is waning. The KA-induced elevation in [GABA] may partly result from increased GAD activity.

412.7

REVERSIBLE ANTICONVULSANT DISPLACEMENT OF BENZODIAZEPINE (BZ) PHOTOLABELLING TO 65 kDa BZ BINDING PROTEIN. W.C. Taft*, A.C. Bowling* and R.J. DeLorenzo (SPON: V.T. Calabrese), Dept. Neurology, Medical College of Virginia-VCU, Richmond VA 23298.

We have identified a 65 kDa benzodiazepine binding protein (BZBP) which photolabels with photoactive BZs in concentrations that produce therapeutic effects on generalized tonic-clonic (GTC) seizures, maximal electric shock (MES)-induced seizures and limitation of spike frequency adaptation (SFA) (Eur. J. Pharm. 35, 97, 1987). BZ photolabelling to BZBP is saturable, stereoselective and displaceable with other photoactive BZs. In this study, we have examined the effectiveness of non-photoactive BZs and other anticonvulsants in displacing BZ photolabelling to BZBP. [^{3}H]Flunitrazepam ([^{3}H]FNZ) was equilibrated with BZBP in the presence or absence of non-photoactive competitor. Quantitation of [^{3}H]FNZ photolabelling to BZBP was performed by SDS-PAGE and autoradiography. Reversible, concentration-dependent displacement of [^{3}H]FNZ photolabelling was observed with diazepam, phenytoin, carbamazepine, medazepam and phenobarbital, drugs which are effective against GTC seizures, MES-induced seizures and SFA. Trimethadione and ethosuximide, drugs which are not effective against GTC seizures, MES-induced seizures and SFA, did not displace [^{3}H]FNZ photolabelling. Thus, the pattern of anticonvulsant displacement of [^{3}H]FNZ photolabelling to BZBP is similar to anticonvulsant efficacy against GTC seizures, MES-induced seizures and SFA. The results suggest that the 65 kDa BZBP may function as a general anticonvulsant binding site.

412.8

INTRACELLULAR EJECTION OF 65 kDa BENZODIAZEPINE BINDING PROTEIN (BZBP) REDUCES SPIKE FREQUENCY ADAPTATION IN AN IDENTIFIED HERMISSENDA NEURON. R.R. Forman, S. Sombati, W.C. Taft* and R. J. DeLorenzo. Dept. Neurology, Medical College of Virginia-VCU, Richmond VA 23298.

Benzodiazepine (BZ) enhancement of spike frequency adaptation (SFA), a mechanism which may underlie BZ effectiveness in suppressing tonic-clonic seizures in mammals, is not mediated by BZ binding to central GABA-BZ or peripheral BZ receptors (McLean and Macdonald, J.Pharm.Exp.Ther. 244, 789, 1988). We have isolated a 65 kDa BZBP which binds BZs in concentrations that enhance SFA (Eur.J.Pharmacol. 35, 97, 1987). In a blind study, we have injected purified BZBP (prepared from rat brain cytosol by sequential affinity chromatography) into LP1, an identified neuron on the dorsal surface of the pedal ganglion of the nudibranch mollusc, Hermissenda, and have evaluated its effects on SFA in response to depolarizing current stimuli. A single ejection of BZBP (15-20 lbs/in^2, 10 s) produced a mean 66% increase in spike frequency observed in response to a 10 s stimulus (n=6 cells). This effect peaked within 5 min of ejection and remained constant for at least 30 min. Control ejections of vehicle alone, BZ photolabelled BZBP, or heat-inactivated BZBP did not produce changes in spike frequency (n=7 cells). Ejection of either BZBP or control solutions had no effect on resting membrane potential. These results suggest that the 65 kDa BZBP may modulate SFA and that BZ binding to this protein may play a role in the regulation of SFA.

412.9

REGULATION OF THE $GABA_A$ RECEPTOR COMPLEX IN BRAIN FOLLOWING LIMBIC SEIZURES PRODUCED BY KAINIC ACID. Patricia Edgar*, Mark A. Bowe and Rochelle D. Schwartz. (SPON: T.A. Slotkin). Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.

Systemic kainic acid (KA) was used to induce limbic epilepsy in rats. The effects of seizure activity on regulation of the GABA receptor/Cl^- channel were determined by measuring GABA receptor-gated $^{36}Cl^-$ flux in rat brain vesicles and binding of [^{35}S]TBPS to "convulsant" sites associated with the GABA receptor/ionophore. Rats were injected with KA (5-20 mg/kg, ip) and sacrificed 2 hrs later. Within this time period initial decreases in locomotor activity followed by catalepsy, wet dog shakes, and finally limbic motor seizures were observed. KA-induced seizure activity decreased maximal muscimol-activated $^{36}Cl^-$ uptake in cerebral cortical synaptoneurosomes by 12% ($p=0.0053, n=9$). The distribution of "convulsant" sites labeled by TBPS was measured using in vitro receptor autoradiography. KA-induced seizure activity caused a significant decrease in TBPS binding in fronto-parietal cortex (15%, $p < 0.02$), inferior colliculus (52%, $p<0.001$), and molecular and granular layers of cerebellum (43%, $p<0.002$, and 52%, $p < 0.001$, resp.) These data suggest that KA-induced limbic motor seizures can decrease the activity of the $GABA_A$ receptor-gated ion channel and convulsant binding to the Cl^- ionophore in specific brain regions.
Supported by NIH grant NS 24577 and PMAF Award to RDS.

412.10

INTRACELLULAR RECORDING FROM NEOCORTICAL SLICES OBTAINED DURING THE GABA WITHDRAWAL SYNDROME. C. Silva-Barrat*, J. Champagnat*, S. Brailowsky, C. Menini* and R. Naquet. Laboratoire de Physiologie Nerveuse, C.N.R.S., 91190 Gif sur Yvette, France.

The interruption of intracortical, chronic GABA infusion gives rise to an epileptogenic process, which has been named the "GABA withdrawal syndrome (GWS)" (Brain Res 442:175,1988). After induction of a GWS (EEG spikes and myoclonus) in vivo, we prepared slices and recorded neurons located in the vicinity of the infused site. Membrane potentials of more than 60mV and Na^+ action potentials of 70-110mV indicated that neurons studied were not injured by the experimental procedure. Electrical stimulation of the underlying white matter induced 15-30mV, 80-150msec paroxysmal depolarization shifts in virtually all neurons, indicative of epileptiform activity. Bath applications of GABA (0.3-10 uM) in these neurons had no effect, while the same dose range was found effective in control slices obtained from non-infused animals. In neurons unresponsive to high doses (100 uM) of GABA, voltage-dependent (non-synaptic) depolarization shifts and bursts of action potentials were induced by depolarizing current injections.

These results indicate a correlation between cortical GABAergic dysfunction and epileptiform activities at the cellular level following increased and prolonged GABA exposure in vivo.

412.11

EFFECT OF LIDOCAINE ON INHIBITION IN THE RAT HIPPOCAMPAL SLICE. B. Esplin and R. Capek. Dept. of Pharmacol. and Therapeutics, McGill University, Montreal, Quebec, H3G 1Y6, Canada.

Depression of inhibitory pathways is assumed to be responsible for the seizures induced by local anesthetics. We studied the effects of lidocaine on inhibition in the hippocampal slice using paired-pulse tests. The population spikes (PSs), evoked by orthodromic stimulation and recorded in the CA1 region, were inhibited by conditioning stimuli, either orthodromic (ortho-ortho) or antidromic (anti-ortho).

Lidocaine (50 to 300 µM) administered by perfusion produced a concentration-dependent depression of the unconditioned PSs. In the anti-ortho test, 100 µM and higher concentrations of lidocaine decreased inhibition. In the ortho-ortho test, reduced inhibition was also seen in most experiments, but 200 µM of lidocaine occasionally enhanced and prolonged the inhibition. No multiple PSs were observed during lidocaine perfusion.

Although these data do not contradict the suggestion that the hippocampus is probably not the site of origin of seizures induced by local anesthetics (Shurr et al., Anesthesiol., 64: 501, 1986), hippocampal disinhibition can play an important role in generalization of seizures induced by lidocaine *in vivo*.

(Supported by the MRC of Canada).

BLOOD/BRAIN/NERVE BARRIER II

413.1

COMPUTER ANALYSIS OF TWO METHODS OF MEASURING BLOOD BRAIN BARRIER TRANSPORT KINETICS Richard A. Hawkins and George A. Oyler*. Dept. Anesthesia, Milton S. Hershey Medical Center, Hershey, PA 17033

A computer model of blood-brain barrier transport, was used to compare the ability of two experimental techniques, the brain uptake index technique and the in situ brain perfusion method, to accurately measure blood-brain barrier transport kinetics. The model allows the experimentally observed values of V_{max} and K_m to be predicted when kinetic parameters are assigned to both endothelial cell membranes. The model considers the effects of two membranes, blood flow, substrate concentration gradients along the length of the capillary, substrate competition, and non-steady state conditions. Two kinetically different systems, glucose and neutral amino acid transport, were evaluated. Our analysis revealed that inaccurate estimates of the K_m and V_{max} can result from both methods. The apparent kinetic constants are critically dependent on a variety of factors including the range of concentations used, the time of the experiment, the endogenous concentration of substrates and the V_{max}-to-K_m ratio. The results of neutral amino acid transport experiments are particularly sensitive experimental time and the concentration range examined. Supported by NS 16737.

413.2

TIME COURSE OF THE PENETRATION OF SYSTEMICALLY ADMINISTERED IgG INTO THE RAT NEONATAL BRAIN PARENCYMA. Roderic H. Fabian*, & Claire E. Hulsebosch+. *Department of Neurology and +Anatomy and Neurosciences and the *+Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas, 77550.

Insight concerning CNS development has been gained by examining the effect of anti-neuronal antibodies on development. We undertook this study to determine whether or not systemically administered xenogeneic IgG crosses readily into the neonatal rat brain parenchyma. Sixteen 2 to 4 day old rat pups were compared to 12 mature rats (150 - 300g) studied previously. Rats were injected intraperitoneally with purified rabbit IgG (1.3 g/kg) or were left uninjected as controls. After 2, 6, 12, 24, and 48 hours, they were deeply anesthetized and perfused, and the neuraxis was removed and examined for the presence of rabbit IgG using a controlled immunohistochemical technique. Rabbit IgG titers were determined using an ELISA technique. Sections from pups injected with rabbit IgG stained diffusely and strongly positive for rabbit IgG after 6 hours, unlike sections from mature rats which showed no staining except for specific cell groups and the circumventricular organs. Controls showed no staining. Rabbit IgG titers in rat pup serum were maximal at 2 hours after injection. This study supports the assertion that systemically administered xenogeneic IgG reaches significantly higher concentrations in the brain parenchyma of the neonatal rat than in the mature rat. Supported by NIH grant NS 11255.

413.3

MONOCLONAL ANTIBODY CROSSING BLOOD-BRAIN BARRIER BINDS BRAIN ACETYLCHOLINESTERASE. S. Brimijoin, M. Balm, P. Hammond, and V. A. Lennon. Depts. of Pharmacology, Immunology and Neurology, Mayo Clinic, Rochester MN 55905.

As a model of autoimmunity to acetylcholinesterase (AChE) adult rats were given single i.v. injections of an equal part mixture of 7 monoclonal anti-AChE antibodies (ZR1-7). Even 2 mg of IgG caused little motor impairment or other typical signs of anticholinesterase intoxication (diarrhea, profuse salivation), but it did cause sympathetic dysfunction. For example, the antibody mixture quickly induced a pronounced and persistent bilateral ptosis. Biochemical abnormalities were also striking. AChE disappeared from plasma. Synaptic 16S and 10S AChE were depleted from diaphragm. Residual oligomeric AChE in muscle was largely complexed with IgG. Immune complexes were unexpectedly abundant in brain. Up to 65% of the AChE in cortex became antibody-bound. The binding was no postmortem artifact, as shown by the time course (steady accumulation over a 3 day period), by the preferential binding of 10S AChE, and by the 30% reduction of cortical AChE activity ($p < 0.001$). Furthermore, although individual antibodies all bound solubilized enzyme, only one bound brain AChE in vivo. This antibody clearly crossed the blood-brain barrier. Additional studies with AChE-antibodies may shed light on some poorly understood disorders of peripheral and central cholinergic systems.
(Supported by NIH Grants NS18170 and NS15057).

413.4

EFFECTS OF Al^{3+} AND Pb^{2+} ON CEREBROMICROVASCULAR $(Na^+ + K^+)$-ATPase AND MUSCARINIC RECEPTROS. M.L. Caspers and P. Grammas*, Dept. of Chem., Univ. of Detroit, Detroit, MI 48221 and Dept. of Pathol., Wayne State Univ., Detroit, MI 48201.

The cerebromicrovasculature plays a critical role in the maintenance of the brain ionic environment and has characteristics that form the basis of the blood-brain barrier (BBB). Muscarinic receptors exist on cerebral endothelium as does the $(Na^+ + K^+)$-ATPase. Circulating neurotoxic agents, including metal ions such as Pb^{2+} and Al^{3+}, may alter receptor and enzyme function in endothelium. Experiments are performed on microvessels (capillaries) obtained from rat cerebral cortices. The effects of Pb^{2+} and Al^{3+} on $(Na^+ + K^+)$-ATPase and muscarinic receptors are evaluated using $[^3H]$-ouabain and quinuclidinyl benzilate (QNB) binding, respectively. Measurement of QNB binding at 0.094 nM and 0.25 nM (basal 206 and 315 fmol/mg, respectively) indicates a 70-85% decrease with either 100μM Pb^{2+} or Al^{3+}. At 1 mM Pb^{2+} or Al^{3+} a pronounced inhibitory effect (90%) on $[^3H]$-ouabain binding (basal 2.42 pmol/mg) is noted. However, at 1 μM Pb^{2+} or Al^{3+} a slight stimulation (20-25%) or $[^3H]$-ouabain binding is demonstrable. In summary, these data suggest that Pb^{2+} and Al^{3+} affect microvascular $(Na^+ + K^+)$-ATPase and muscarinic receptors and that these changes may constitute the underlying mechanism for CNS dysfunction associated with these metal ions. (Supported in part by USPHS HL 23603, ANR Pipeline Co., and J. Rose).

413.5

TRANSFER COEFFICIENTS FOR UPTAKE OF Ga-67, Cd-109, AND Pb-203 INTO BRAIN AND CEREBROSPINAL FLUID (CSF). V.A. Murphy, Q.R. Smith, S.I. Rapoport. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

Metals such as Pb and Cd have been shown in numerous investigations to be neurotoxic. However, little is known of how these metals cross the blood-brain barrier (BBB) and enter the brain. Transfer coefficients (K) for uptake of Ga-67, Cd-109 and Pb-203 into brain and CSF were determined in adult rats after i.v. administration of each tracer (J. Neurochem. 36: 1463, 1981 and 46: 1732, 1986). K's are listed for CSF, parietal cortex (PC), hippocampus (HC), midbrain-colliculi (MBC), and pons-medulla (PM) (Ca-45 K's are included for comparison):

	n	Mean (SEM) K (nl/g per s)			
		Ca-45	Pb-203	Cd-109	Ga-67
CSF	3-6	170 (10)	20.7 (1.4)	0.5 (0.1)	0.48 (0.06)
PC	8	5 (1)	23.1 (1.2)	6.0 (0.2)	0.25 (0.06)
HC	8	15 (3)	19.0 (3.5)	4.5 (0.1)	0.38 (0.04)
MBC	8	10 (1)	23.7 (4.8)	6.0 (0.3)	0.31 (0.08)
PM	8	14 (2)	28.0 (4.0)	6.3 (0.1)	0.33 (0.06)

Unbound plasma tracer (% of total) determined by ultrafiltration was 9.9% for Pb-203, 1.1 for Cd-109, and 1.1 for Ga-67 (Ca is 50). The results demonstrate restricted uptake of the metals across the BBB due to low passive permeability of the free metal or metal complexes. In addition, the rate of entry across the choroid plexus (CSF) compared to that of the cerebral capillaries (parietal cortex) varied among the metals.

413.6

KINETIC ANALYSIS FOR L-CARNITINE TRANSPORT IN THE RABBIT CHOROID PLEXUS. C.S. Kim* (SPON: C.G. Lineberry). Lab of Cellular and Molecular Pharmacology, NIEHS/NIH, Research Triangle Park, NC 27709.

All kinetic studies were conducted with the lateral or fourth ventricular choroid plexus (LVCP; FVCP) of the rabbit. At L-carnitine concentration of 10 μM, the uptake was increased linearly for the first 10 min. Steady state levels were reached by 30 min. with tissue concentrations (T/M) ranging from 20 (FVCP) to 34 fold (LVCP) greater than medium concentrations. Initial uptake rates were determined from 5 min. uptake values at 37 °C. As the L-carnitine concentration of the medium increased from 0.01mM to 1.0mM, the T/M ratios (mean ± S.D.) fell from 3.6 ± 1.2 to 1.2 ± 0.2 (FVCP) and from 6.1 ± 0.9 to 1.5 ± 0.2 (LVCP), respectively, suggesting saturation of the carrier-mediated transport process. Kinetic analysis obtained from the saturable component of the transport process yielded apparent K_m of 37 μM (FVCP) and 35 μM (LVCP) and V_{max} of 19 (FVCP) and 23 nmol/ml/min (LVCP), respectively. Ouabain inhibited the uptake by 47% (FVCP) and 42% (LVCP) and hypothermia (0 °C) produced inhibition by 96% (FVCP) and 97% (LVCP), respectively. However, neither organic acid inhibitor, probenecid nor tyrosine inhibited L-carnitine transport by the choroid plexus.

413.7

FACILITATED TRANSFER OF L-KYNURENINE ACROSS THE BLOOD-BRAIN BARRIER. S. Fukui*, R. Schwarcz, S.I. Rapoport, and Q.R. Smith. Lab. of Neurosciences, NIA, NIH, Bethesda, MD 20892 and Maryland Psychiatric Research Center, Baltimore, MD 21228.

L-Kynurenine (KYN), a tryptophan metabolite, serves as precursor to two key neuroactive substances, quinolinic acid and kynurenic acid. Although formed primarily in the periphery, KYN can cross the blood-brain barrier and be taken up into brain after peripheral administration (Gal & Sherman, 1980). To determine the mechanism of transfer, we examined the concentration dependence and sensitivity to inhibition of KYN transport across the blood-brain barrier. Transport was measured in pentobarbital-anesthetized rats using the in situ brain perfusion technique of Takasato et al. (1984). In the absence of competing amino acids, the transfer coefficient for KYN uptake into brain at tracer concentrations equaled 3 x 10^{-3} ml/s/g, a value 30-times greater than that expected for transport by passive diffusion. KYN influx into brain was saturable with a estimated Vmax of 5.9 x 10^{-4} umol/s/g and a Km of 0.19 umol/ml. Addition of 10 umol/ml leucine to perfusate containing KYN decreased KYN influx into brain by 85%. Similarly, addition of 10 umol/ml KYN to perfusate containing leucine decreased leucine influx into brain by 96%. These results show that KYN is transported into brain by the large neutral amino acid carrier at the brain capillaries. (Supported by USPHS Grant NS16102 to RS).

413.8

SELECTIVE CONTROL OF CELL WATER DURING ACUTE HYPERNATREMIA IN RAT CEREBRAL CORTEX. C. Nicholson, H.F. Cserr, M. DePasquale*, C.S. Patlak*, and M.E. Rice. Physiology and Biophysics, NYU Medical Center, NY, NY 10016.

Total brain water is regulated during acute hypernatremia based in part on tissue uptake of Na and Cl (Cserr et al., Am. J. Physiol. 253:F522, 1987). We have studied the separate regulatory responses of extracellular water (ECW) and intracellular water (ICW) to this osmotic stress in the cerebral cortex of anesthetized Sprague-Dawley rats. Plasma [Na] was elevated by a single ip injection of NaCl (1.25 M, 2 ml/100 g), and changes in plasma and cortex were monitored for 2 hours. ECW was measured in situ using TMA^+ and ion-selective microelectrodes (Nicholson & Phillips, J. Physiol. 321:225, 1981). Total tissue water and electrolytes were determined, in a separate series of experiments, as functions of plasma [Na]. ICW could then be estimated as the difference between total water and ECW. ECW and ICW (gm water/gm dry wt brain, mean +SE, N=52) in control cortex were 0.86±0.02 and 2.93±0.02, respectively. ECW decreased by an average of 60% after 2 hours of hypernatremia, whereas ICW remained stable. Plasma [Na] increased from 141 to 172 meq/liter, tissue Na content by 25%, and Cl content by 40%. The decrease in ECW, together with simultaneous increases in tissue Na and Cl content, indicate that a shift of extracellular fluid into brain cells contributes to regulation of ICW during acute hypernatremia. Supported by NIH grants NS-11050 and NS-13742.

413.9

USE OF BUMETANIDE AND FUROSEMIDE TO TEST FOR NaK2Cl COTRANSPORT IN THE ISOLATED RAT CHOROID PLEXUS. C. Johanson, D. Bairamian, J. Parmelee, S. Sweeney and M. Epstein. Dept. of Clin. Neurosci., Prog. in Neurosurg., Brown Univer. and R.I. Hosp., Providence, RI 02902.

The regulation of pH, volume and secretion in the choroid plexus-CSF system is becoming clearer as the nature of Cl transport in choroid plexus (CP) is elucidated. Cl-HCO3 exchange in the CP is established; less clear is the possible functional role of NaK2Cl cotransport. We tested for cotransport by analyzing the kinetics of Cl-36 and Rb-86 (potassium) uptake by lateral ventricle CP incubated in artificial CSF (37 C). Choroidal tissues were excised from adult Sprague-Dawley rats (Metofane anesthesia) and preincubated for 20 min in CSF (with drug but without tracer) before transfer to tracer-containing CSF. The rapidity of Cl uptake, i.e., 1-2 min for steady state, suggest carrier-mediated uptake "uphill" into the choroid epithelium. Bumetanide and furosemide, inhibitors of NaK2Cl cotransport, reduced by 25-30% the transport of Cl-36 into the plexus incubated in CSF with drugs (10^{-6} to 10^{-4} M). Over the same concentration range, these loop diuretics decreased tracer Rb (K) accumulation by up to 25%; such findings were corroborated by choroidal tissue analysis of stable K (by flame photometry). The ability of bumetanide to suppress both Cl and K transport in the CP is evidence for cotransport, and suggests its usefulness as a probe in CNS (blood-brain barrier) and CSF transport models.

413.10

THE EFFECT OF LARGE NEUTRAL AMINO ACIDS ON THE PHARMACOKINETICS OF LEVODOPA IN CSF J.P. Hammerstad, W.R. Woodward, P. Gliessman,* T. Trotman* and J.G. Nutt. Depts. of Neurology and Biochemistry, Oregon Health Sciences Univ. and The Oregon Regional Primate Research Center, Portland, OR 97201.

The transport of levodopa from blood to brain occurs via the large neutral amino acid (LNAA) carrier system. Because the Km of LNAA transport approximates the plasma concentration of LNAA's, competitive inhibition of levodopa influx into brain may result from physiologic increases in plasma levels after ingestion of protein. This may account for the effects of meals on the response to levodopa in patients with Parkinson disease (PD) who exhibit the fluctuating motor response ("on-off"). In PD patients with "on-off" an oral load of a LNAA blocks the clinical response to a therapeutic level of levodopa maintained by a constant I.V. infusion. Using a similar approach in rhesus monkeys, we examined the effect of a leucine load given I.P. on the disposition of levodopa and other amino acids in cisternal CSF as a more direct indication of the effect of LNAA's on the influx of levodopa into brain. Leucine loading produced changes in CSF levels of LNAA and levodopa as well as other amino acids. An unexpected result was a 4-6 fold increase in CSF glutamate levels.

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413.11

PARAVASCULAR FLUID MOVEMENT AFTER MICROEMBOLIC DISRUPTION OF THE BLOOD-BRAIN BARRIER. **O.R. Blaumanis* and M.L. Rennels. Departments of Neurology and Anatomy, Univ. of Md. Sch. of Med., Balto., MD 21201.**

Embolization of the rat brain with polystyrene microspheres (MS) results in focal opening of the blood-brain barrier (BBB). At these sites, leakage and spread occurs of plasma constituents and tracers such as horseradish peroxidase (HRP). To elucidate the mechanisms involved, pentobarbital anesthetized rats received unilateral intracarotid injections of 15 μm MS (5,000 - 100,000). Twenty min. to 24 hrs. later, HRP was given i.v. (60 mg/kg). After 10-20 min. HRP circulation, the rats were perfused with aldehydes and brain sections were processed for tracer localization by light and electron microscopy (EM). Sites of HRP leakage were always associated with arterioles occluded by MS (even a single MS causes local BBB disruption). Around these vessels, tracer occupied enlarged perivascular spaces (PVS) and basal laminae (BL) of arterioles, capillaries and venules. Diffuse gradients of HRP reaction product extended into the parenchyma. EM confirmed the dense accumulation of tracer in BL and PVS, with variable infiltration of the local extracellular spaces. Cellular uptake of HRP was limited to perivascular macrophages and intraperenchymal microglia. In addition, there was variable swelling of astrocytic processes. Microembolization does not lead to capillary stasis and there is no ultrastructural evidence of ischemic neuronal damage. **(NIH Grant No. NS 16332).**

413.12

A RAPID, SENSITIVE METHOD FOR QUANTITATION OF ESTRADIOL CONJUGATES IN BRAIN TISSUE AFTER ADMINISTRATION OF A BRAIN-ENHANCED DELIVERY SYSTEM FOR ESTRADIOL. M. Rahimy* and J.W. Simpkins. University of Florida, Gainesville, FL 32610.

Brain-enhanced delivery of Estradiol (E_2) with an E_2 chemical delivery system [i.e. estradiol 17-(1,4-dihydrotrigonellinate)] creates a problem for separating and quantitating brain levels of the active E_2 in the presence of excess concentrations of the inactive conjugated forms of the drug. We have developed a 4-step method that permits the rapid quantitative analysis of E_2 and its conjugates. The first step uses a water:acetone (50:50, v:v) solvent system to extract the conjugate from biological tissues (e.g. rat brain); second, the conjugates are hydrolyzed in 1N NaOH; third, solid phase extraction (SPE) with C_{18} reverse phase column is used to isolate E_2; and the final step is the RIA of E_2. The application of this procedure to biological tissues was assessed using rat brain homogenates. Recovery of the E_2, after solvent extraction and SPE chromatography, is 101 ± 4.5% while recovery of the E_2 conjugate is 64 ± 2% with greater than 80% hydrolysis of the conjugate. Since the solvent extraction, hydrolysis, and SPE steps can be completed for 100 samples in one day, this procedure represents a rapid, reliable and practicable method for the quantitation of E_2 and its conjugates in tissue samples (Supported by NIH HD 22540).

413.13

CEREBROVASCULAR PERMEABILITY IN EXPERIMENTAL HYPERTENSION. P.A. Grady. Univ of Maryland Sch of Med.,Balt, MD 21201.

Cerebrovascular permeability remains unaltered in most physiological states due to an intact blood brain barrier. This barrier has been shown to be breached under conditions of acute hypertension, but what occurs in chronic hypertension is less clear. This study tests the effect of chronic hypertension on cerebrovascular permeability.

Goldblatt hypertensive rats were monitored for 3 months. Blood pressures ranged from 135-165 mm Hg systolic arterial pressure. After 3 months, these rats and controls were anesthetized with chloral hydrate 100 mg/kg ip.Horseradish peroxidase(HRP) Sigma Type II or VI was injected iv and allowed to circulate for periods of 5, 10, or 20 minutes. Animals were perfused with fixative. Brain sections were incubated with diaminobenzidine and tetramethylbenzidine and examined at the light microscopic level.

Patchy areas of reaction product were scattered diffusely across gray and white matter areas. TMB incubated sections showed increased staining over the rostral portions of the cerebral hemispheres, basal forebrain and pontine areas of brainstem predominently.

The results of this study suggest that there is an increased cerebrovascular permeability under conditions of chronic hypertension which allows for the passage of macromolecules into brain parenchyma. These findings provide a basis for longterm changes in the brain in chronic hypertension.(Supported in part by PHS NS 16332)

413.14

DUAL ROLE OF ENDOTHELIAL CELL TUBULES IN BLOOD-BRAIN BARRIER INJURY. A. S. Lossinsky and M. J. Song*. NYS Inst. for Bas. Res. in Devel. Disabil., S.I., NY 10314 and NIH Res. Cent. for HVEM, NYS Dept. of Health, Albany, NY 12201

The present studies extend our exploration of the ultrastructural features of tubular profiles manifested in endothelial cells (ECs) following blood-brain barrier (BBB) insult. We have reassessed several of our previous experimental paradigms in an attempt to understand the functional role of these tubular profiles. Previous studies have indicated that the tubules represent either: (1) lysosomal-directed structures for the metabolic requirements of the cell, or (2) serve as purported conduits for trans-EC macromolecular transport. In several experimental murine models in which the BBB was compromised, employing conventional transmission or high voltage electron microscopy, we observed horseradish peroxidase-positive, conduit-like structures, directly connecting luminal and ablumenal surfaces in the thinnest portions of the EC. In wider EC regions, tubules were observed in the proximity of, or bound to secondary lysosomes or multivesicular bodies. Inflammatory cells (ICs) by scanning microscopy were often observed attached to parajunctional EC regions. Our results suggest a dual functional role of these tubules for macromolecular transfer and transport and also implicate a possible mechanism for IC attachment and eventual migration across the injured BBB. Supported by NYS OMRDD and NIH Grant # RR01219.

413.15

REGULATION OF CEREBROVASCULAR GUANYLATE CYCLASE BY NEUROHUMORAL AGENTS. P.Grammas*, F. Giacomelli*, C. Diglio* and J. Wiener* (SPON: H. Goldman).Dept. of Pathol. Wayne State Univ., Detroit, MI 48201.

Regulation of cerebromicrovascular endothelial cell function is critical to the maintenance of the blood-brain barrier (BBB). There is evidence from peripheral tissues that atrial natriuretic factor (ANF) modulates fluid volume and that this is mediated by guanylate cyclase activity. Norepinephrine and AII have been shown to affect BBB permeability under physiologic and pathologic conditions. In the present study, control of guanylate cyclase activity in cerebral microvessels (capillaries) by ANF, angiotensin II (AII) and the β- adrenergic agonist phenylephrine (PE) was evaluated. Guanylate cyclase activity was measured in isolated rat cerebral capillaries by radioimmunoassay for cGMP. Untreated microvessels yielded 0.058 ± 0.018 pmol cGMP/mg protein. ANF (0.95μM) increased cGMP about 10 fold to 0.59 ± 0.16 pmol/mg protein. Interestingly, while incubation of microvessels with AII (1μM) or phenylephrine (1μM) did not significantly change the level of cGMP (0.06 and 0.075, respectively), both AII and PE greatly potentiated the stimulation of guanylate cyclase by ANF (1.17 ± 0.4 and 1.09 ± 0.3 pmol cGMP/mg protein,) respectively. These data suggest that interaction of neurohumoral receptors in cerebral endothelium may control cyclic nucleotide formation and that this interaction might regulate cerebrovascular permeability to ions and water.

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413.16

CYCLO-PHOSPHAMIDE SHORTENS THE LATENCY FOR RADIATION INDUCED BBB BREAKDOWN M. P. Remler and W. Marcussen* (SPON: A. Gabor) Dept. of Neurology, VAMC, Univ. of California, Davis, Martinez, CA, 94553

Ionizing radiation is known to lower the blood brain barrier (BBB) after a dose dependent latency. Just prior to irradiation, the rats were treated with the radiation sensitizers Chlorpromazine 37.5 mg/kg, Cisplatin 5 mg/kg, Cyclophosphamide 100 mg/kg, Acyclovir 100 mg/kg, Bleomycin 3 mg/kg, Doxorubicin 10 mg/kg, Actinomycin 0.75 mg/kg, and Dactinomycin 0.12 mg/kg. Rats were irradiated with 20 Gy of alpha particles in a single dose focused to a volume of 0.5 cc in the center of the left hemisphere. Breakdown of the BBB was detected in the irradiated portion of the brain by the presence of staining following Evans Blue dye injection. All except cyclophosphamide showed no reduction of the latency of 220 days expected for BBB breakdown following 20 Gy. Of four rats test with cyclophosphamide, one showed no BBB breakdown at 106 days while three showed breakdown at 134, 148 and 169 days. The left hemispheres with lowered BBBs were histologically normal as were the unirradiated control right hemispheres.

This research was supported by the Veterans Administration and the USPHS NIH Grant No. NS 17777.

413.17

BLOOD-BRAIN BARRIER (BBB) CHANGES IN RATS AFTER INTRACEREBRAL INJECTION OF HUMAN RECOMBINANT INTERLEUKIN-2 (rIL-2). RG Watts*, JL Wright*, LL Atkinson* and RE Merchant* (SPON: JW Bigbee). Med Coll of VA, Richmond, VA 23298.

Clinical trials utilizing intracerebral (IC) injection of rIL-2 and lymphokine-activated killer cells to treat brain tumor have reported temporary exacerbation of neurological deficit and lethargy in most patients. The current study examined the histopathological effects of a single 5ul injection of rIL-2 (12,000 U, CETUS), its excipient or saline into the right parietal lobe of rats. Animals were sacrificed at various times up to 8 days by transcardiac aldehyde perfusion (1h following IV injection of horseradish peroxidase (HRP)) and brains were processed for light and electron microscopy. Histochemical findings at 4h, 12h and 24h post-injection showed traumatic BBB disruption with no differences between injections of rIL-2, excipient or saline. HRP extravasation persisted at 3 and 8 days only in animals injected with rIL-2. In addition, only rats receiving rIL-2 demonstrated increased cellularity at the injection site with leukocytic infiltration, perivascular cuffing and localized edema which was evident at 24h and continued to increase over an 8 day period. Our results suggest that an IC injection of rIL-2 alters the integrity of the BBB directly and/or potentiates and sustains cellular events responsible for barrier disruption following traumatic injury to the brain.

413.18

HOST BLOOD-BRAIN BARRIER PERMEABILITY IS UNALTERED BY INTRAPARENCHYMAL FETAL NEURAL GRAFTS. M.S. Grady, M.J. Geist*, D.O. Maris*. Dept. of Neurol. Surgery, U. of Washington, Seattle WA 98104.

The permeability of the blood-brain barrier (BBB) may be important in the immunologic response of the host CNS to grafted tissue. There is evidence (Rosenstein, *Science* 235:772-774, 1987) that vasculature established in block grafts of fetal neural tissue no longer maintains characteristics of normal BBB despite the fact that the barrier is mature (in terms of its permeability to certain molecules) even in the embryo. However, fetal neural cell suspensions injected into host brain parenchyma may not cause this disruption of the BBB, as vascular growth may originate from the host rather than the donor.

Anesthetized adult male Sprague-Dawley (S-D) rats received grafts of E15 S-D forebrain. The donor tissue was dispersed into a cell suspension using trypsin (Schmidt et al., *Brain Res* 218:347-356, 1981) then injected stereotactically into the rostral corpus callosum. The opposite side received a vehicle injection. Survival periods of 14 days (n = 3), 30 days (n = 2), 90 days (n = 4), and 180 days (n = 4) were studied. One hour before the animals were sacrificed, 5 mg/kg of HRP type VI (Sigma) was injected i.v., after 1 mg/kg Benadryl (diphenhydramine) was given i.v. to prevent anaphylaxis. The anesthetized animals were transcardially perfused with 1% paraformaldehyde, 1.25% glutaraldehyde, and 50 micron coronal sections taken on a vibratome. Thionin stain acetylcholinesterase (AChE) histochemistry and HRP histochemistry (Mesulam method) were performed.

HRP is normally excluded from brain parenchyma where the BBB is intact. The BBB was impermeable to HRP at the control site at all time points. No graft showed HRP leakage into the parenchyma except one 14 day graft that was contaminated by non-neural tissue. All grafts formed a solid cluster of cells and demonstrated AChE positive cells and neurons as suggested by Nissl substance. Vascular distribution in the grafts was extensive, with no apparent difference between host or graft vessels.

Vasculature established in neural cell suspension grafts maintains certain BBB qualities such as impermeability to medium molecular weight proteins such as HRP.

413.19

ANIONIC PROPERTIES OF ENDOTHELIAL CELLS AFTER MODERATE CONTUSIVE INJURY TO THE RAT SPINAL CORD. L.J. Noble (SPON: M.K. Floeter). Dept. of Neurology, School of Med., UCSF, San Francisco, CA 94122.

After spinal cord injury, abnormal barrier permeability to the tracer horseradish peroxidase (HRP) and the distribution of cationized ferritin (CF), a marker of anionic sites, were examined in the spinal cord at the ultrastructural level. In control (laminectomized) animals, vessels remained impermeable to HRP. Although the luminal surface of microvessels was evenly labelled with CF, vesicle formation along the luminal front was associated with partial thinning and/or loss of anionic sites. In initial stages of vesicle formation (prior to stalk formation) anionic sites appeared reduced along the invaginated membrane. With formation of a stalk, negative sites were present along the neck of the stalk with fewer CF molecules in the vesicle proper. CF was absent in vesicles which no longer maintained continuity with the plasma front or which contacted the abluminal front.

After injury, the distribution of anionic sites, relative to luminal vesicle formation, was similar to that observed in laminectomized animals. However, there was reduced CF binding along the luminal plasmalemma. This loss of surface charge properties was typically associated with edematous tissue as indicated by enlarged perivascular spaces, swollen astrocytic foot processes and abnormal vascular permeability to HRP.

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413.20

NERVE SEGMENTS FORMED IN SILICONE TUBES RECONSTITUTE A PERINEURIAL BUT NOT A VASCULAR ENDONEURIAL PERMEABILITY BARRIER. N. A. Azzam, A. A. Zalewski and L. R. Williams. Lab. of Neuronal Growth and Regeneration, NINCDS, NIH, Bethesda, MD 20892 and CNS Diseases Research (L.R.W.), The Upjohn Co., Kalamazoo, MI 49001

Peripheral nerves fibers of adult mammals can regenerate through silicone tubes wherein they reform a new nerve segment. In the present study we examined these segments to determine whether endothelial and perineurial sheath cell permeability barriers were also reconstituted. Normally these barriers, via tight intercellular junctions, regulate the movement of macromolecules into the endoneurium from inside and around a nerve. Adult rats had their sciatic nerve cut bilaterally, and the proximal and the distal ends sutured into 10 mm long tubes that were filled with saline. One tube was removed after one month, a time when a cable of tissue extended through the entire tube. The permeability of the blood and perineurial barriers was examined, 4-9 months postoperatively, with the tracer horseradish peroxidase (HRP; M.W. 40,000). The reformed nerve segments, enclosed in or free of the tubes, contained remyelinated axons, blood vessels and perineurial-like cells. However, in contrast to normal or distal regenerated nerve, axons were mini-compartmentalized by perineurial-like cells. All the regenerated blood vessels were excluded from the endoneurium of the mini-compartments, and they were profusely permeable to HRP. The extruded HRP did not penetrate the regenerated perineurial sheaths and enter the environment of the nerve fibers. Our results indicated that a reformed nerve segment contains a perineurial but not an endoneurial vascular permeability barrier. How and why vessels were excluded from the endoneurium and why previously tight endoneurial vessels became permeable requires investigation.

414.1

ROLE OF CALCIUM DEPENDENT INFLUX IN NEURONAL DIFFERENTIATION: A PERIOD OF SPONTANEOUSLY ELEVATED INTERNAL CALCIUM IS CORRELATED WITH A PERIOD OF SENSITIVITY TO EXTERNAL CALCIUM. Janet Holliday and N.C. Spitzer. Dept. of Biology, University of California, San Diego, La Jolla, CA.

Calcium dependent action potentials (APs) can be evoked from Xenopus spinal neurons cultured from neural plate stage embryos from the time they can first be recognized in culture. As the neurons mature during the first day, the ionic dependence of this AP shifts from calcium to sodium (Spitzer and Lamborghini, 1976). Growth of cells under conditions which would prevent calcium entry produces alterations in normal patterns of differentiation (Bixby and Spitzer, 1984; Henderson, Smith and Spitzer, 1984). These studies suggest that calcium influx may have a significant role in development.

The role of spontaneous calcium influx was investigated by monitoring changes in the levels of intracellular calcium during the first day in culture using the fluorescent calcium indicator, fura-2. Spontaneous elevations in internal calcium concentration are evident in the largest percentage of cells during the 6th to12th hours in culture. This is the period during which calcium dependent APs can be evoked. This is also the period which exhibits the highest sensitivity to the removal of external calcium. When normal culture medium is exchanged for calcium free medium during this period, both neurite length and neuron-myocyte contact levels are altered in the same manner as in cultures which have developed in the absence of calcium for the entire growth period of 24 hours. Spontaneous elevations of calcium are not observed in cells bathed in hyperpolarizing medium containing calcium channel blockers or in the absence of external calcium. Thus the elevation of internal calcium is likely to be due to calcium influx. Many of the cells which score as spontaneously active proceed to develop into well differentiated neurons. JH is a fellow of the USPHS; supported by NS15918 to NCS.

414.2

IN VIVO ELECTRICAL DIFFERENTIATION OF EMBRYONIC CHICK MOTONEURONS. D.P. McCobb and K.G. Beam. Physiology Dept., Colorado State Univ., Fort Collins, CO. 80523.

We have used the patch clamp to study the development of intrinsic electrical properties of limb motoneurons isolated from embryonic chicks ranging in age from 4 days (E4, HH stage 20-21) to 11 days (E11, HH stage 37). During this important period hindlegs form from rudimentary limb primordia and 50% of the motoneuronal pool undergoes cell death. Motoneurons were identified by retrograde labelling with the carbocyanine dye DiI. Action potentials and voltage dependent currents were measured 1d after dissociation using "whole-cell" current- and voltage-clamp recordings. We report that the majority of chick limb motoneurons are able to fire overshooting action potentials by E5, and virtually all are by E11. The early develoment of excitability can be attributed at least in part to the early rise in Na+ channel expression; Ina+ increases dramatically between E4 and E6, and only moderately between E6 and E11. Ca++ channel expression is maturing over this span as well. Beginning at E4 T, N, and L -type Ca++ currents are distinguishable. By E11 N and L current expression has dramatically increased, while T current has dramatically decreased, suggesting that N and L currents will be expressed in the mature motoneuron, whereas T current expression may be restricted to early stages in differentiation. We are exploring the hypothesis that electrical differentiation of motoneurons plays a deciding role in the onset of motoneuronal cell death.

Embryonic Age	4 days	5 days	6 days	11 days
% Excitable cells	-	66.7 %	76.9 %	100 %
Mean I_{Na}+ (pA/pF)	57.8	63.3	93.1	108.0
Mean I_{Ca}++ (pA/pF)				
T -type	3.64	-	0.955	0.483
N -type	2.83	-	3.73	5.72
L -type	1.72	-	4.87	6.67

This study was supported by NIH grants NS 07383 to DPM and NS 24444 to KGB.

414.3

ONTOGENESIS OF CALCIUM CHANNELS IN A RODENT BRAIN UTILIZING THE XENOPUS OOCYTE ASSAY. *R.W. Cohen and C.D. Hull. Mental Retardation Research Center, University of California, 760 Westwood Plaza, Los Angeles, CA 90024.*

One method for assessing ontogeny of voltage-dependent calcium channels is by extracting mRNA from brains of different postnatal ages and injecting mRNA into oocytes of Xenopus laevis. Several investigators have shown that exogenous mRNA from rat brains leads to expression of additional Ca^{2+} channels in the oocyte, which activate calcium-dependent Cl^- channels (Leonard, et al., 1987; Umbach & Gundersen, 1987). Using voltage clamp techniques, a current record for the Ca^{2+} channel was established by clamping the oocyte at -80 mV and stepping from -40 mV to +40 mV in the presence and absence of cadmium. After subtraction of Ca^{2+} and Cd^{2+} currents, oocytes containing brain mRNA of 15-24 day old mice have current levels similar to adult rat levels of 75 nA (Umbach and Gundersen, 1987). Similarly measured currents of oocytes injected either with 1-5 day old mouse brain mRNA or water (controls) have much lower values. By applying serotonin to the oocyte bath, a transient outward current was seen only in mRNA-injected oocytes regardless of mouse age. This current has been shown (Dascal, et al., 1986) to involve Cl^- channels (probably by releasing internal Ca^{2+} stores), suggesting that Cl^- channels are present at very early ages. These observations agree with recent studies on development of Ca^{2+} channels using different assays (cf. Kazazoglou, et al., 1983).

414.4

PATTERNS OF TRANSIENTLY EXPRESSED ACETYLCHOLINESTERASE ACTIVITY IN DORSAL THALAMUS OF DEVELOPING RODENTS: SPECIES COMPARISONS. H. Poon*, R.T. Robertson and J. Yu (SPON: E. Davis). Depts.of Anatomy and Neurobiology and Physical Medicine and Rehabilitation, University of California, Irvine, CA 92717.

Previous research has demonstrated patterns of transiently expressed acetylcholinesterase (AChE) histochemical staining in the dorsal thalamus and cerebral cortex of developing rat pups. This transient AChE activity appears to be found within thalamocortical neurons of the ventral basal, dorsal lateral geniculate, and ventral medial geniculate nuclei. The temporal domain of transient AChE expression corresponds well to the time of development of thalamocortical connections, suggesting that transient AChE may play a role in thalamocortical development (Robertson, Neurosci. Lett., 75:259, 1987). The present studies were undertaken to determine whether transient expression of AChE in the thalamus is a common characteristic of developing rodents.

Experiments were performed on rats, mice, gerbils, hamsters and guinea pigs. Adult animals and infants 0-25 postnatal days of age were used. Animals were perfused with aldehydes and frozen sections were processed for AChE histochemistry.

Developing animals of all species tested showed intense and transient AChE histochemical activity of the ventral basal complex. The adult ventral basal complex of all species displayed minimal AChE activity. Infants of all species showed greater AChE activity in the ventral medial geniculate nucleus than did adults, although only the infant rat displayed very intense AChE activity. The dorsal lateral geniculate nucleus of adults of all species displays moderate AChE activity. Infant mouse and hamster show slightly more intense staining, while the infant rat shows the most intense transient AChE staining in the lateral geniculate.

These data demonstrate that transient expression of AChE activity is a phenomenon common to a variety of rodents, although aspects of the transient staining appear to vary between species.

Supported by NSF grant 87-08515 and NIH grant NS-25674.

414.5

INSULIN, INSULINLIKE GROWTH FACTOR-I, AND NERVE GROWTH FACTOR STIMULATE NEURITE FORMATION IN RAT SPINAL CORD CULTURES. G.W. Glazner* and D.N. Ishii Physiology Dept., Colorado State Univ., Ft. Collins, CO 80523.

We have previously shown that insulin and insulinlike growth factors (IGFs) (Recio-Pinto et al. J. Neurosci. 6: 1211, 1986) share with nerve growth factor (NGF) the capacity to support neuron survival and stimulate neurite growth in cultured sympathetic and sensory cells. Here we investigated whether these neurotrophic responses could be elicited in spinal cord cultures as well. Embryonic 17-day-old rat spinal cords were dissociated by gentle trypsin treatment followed by passage through several sieves. The single cell cultures were plated on polylysine coated dishes in 1:1 Ham's F-12/DMEM mix containing 4% serum. About 25-30% of the plated neurons were responsive to insulin. The neurite formation response had an ED50 of about 2 nM for insulin and 1 pM for NGF. The number of neurons with neurites and the average length of neurites were increased. High doses of insulin (1 uM) combined with NGF (0.4 nM) did not significantly increase neurite outgrowth at 3 days over insulin (1 uM) alone. IGF-I (1 nM) also enhanced neurite growth. These data indicate that insulin, IGFs, and NGF may be important physiological regulators of neurite formation and play a role in the development of the spinal cord. (Supported in part by NIH grant RO1 NS24327).

414.6

GAP JUNCTIONS DURING NEOCORTICAL DEVELOPMENT: NORTHERN BLOTTING AND IMMUNOCYTOCHEMISTRY. C.C.G. Naus, D. Feinstein and G.M. Kidder*. Departments of Anatomy and Zoology, University of Western Ontario, London, Canada; Research Institute of Scripps Clinic, La Jolla, CA.

The developmental appearance of gap junctions was examined during postnatal development of the rat neocortex. A cDNA specific for the gap junction mRNA (connexin32) was used to probe Northern blots of total RNA isolated from the neocortex of rats at postnatal day 4, 7, 11, 15, 19 and 25. From postnatal day 4 to 15, very high levels of gap junction mRNA are detectable. By postnatal day 19, a sharp decline occurs in the level of this mRNA. In order to examine the distribution of gap junctions at the cellular level, sections of neocortex at these same developmental times were immunocytochemically stained with a polyclonal antibody to connexin32. Immunoreactivity for gap junctions is more abundant in the upper layers of the developing neocortex, particularly in the marginal zone and cortical plate. The cellular distribution of the mRNA for connexin32 is being examined using in situ hybridization. In addition, Northern blot analysis of mRNA isolated from neuronal and glial cultures should reveal the cellular origin of this mRNA. These results indicate that gap junctions are regulated during neocortical development. (Supported by MRC and NSERC).

414.7

CLONING OF C. ELEGANS AND DROSOPHILA cDNA CLONES THAT HAVE HOMOLOGY TO MAMMALIAN GAP-43. S.C. Ng, L.A. Perkins, G.L. Conboy*, N. Perrimon* and M.C. Fishman. Departments of Medicine, Neurology, and Genetics, Harvard Medical School, Mass. General Hosp., and Howard Hughes Medical Institute

GAP-43 is believed to be involved in neuronal remodeling of development and regeneration. Since the human and rat cDNA sequences are nearly identical (Ng et al., Neuron 1, 1988), and there is hybridization with DNA from other species (Nedivi and Skene, Soc. Neuro. Abstr., 1987), there may be evolutionary conservation of part of the protein. In order to pursue genetic analysis of GAP-43 function, as well as to identify domains conserved through evolution, we have isolated Drosophila and C. elegans GAP-43-homologous cDNA and genomic clones by using reduced stringency hybridization with the rat GAP-43 cDNA as a probe. The nucleotide sequence of one region is especially well conserved between rat, Drosophila, and C. elegans. In Drosophila there is one prominent transcript that is developmentally regulated. By in situ hybridization to the developing fly embryo we have found diffuse expression in the cellular blastoderm, subsequent expression within specific cellular compartments, and, ultimately, at the end of embryogenesis, expression restricted to the ventral nervous system and its overlying glia. We have identified the chromosomal localization of both the Drosophila and nematode GAP-43-homologous genes in order to facilitate mutational analysis.

414.8

DEVELOPMENT OF NEUROFILAMENT IMMUNOREACTIVITY IN THE PARIETAL CORTEX OF THE MOUSE. M.E.Lickey, A.J.Sprute*, A.E. Flavell*, J.I.Espinoza*, S.C.Johnson* & R.A.BreMiller*, Inst. of Neurosci., Univ. of Ore., Eugene, OR 97403.

It has been suggested that neurofilaments (NF) contribute to the stabilization of neuron shape (Willard et al., 84; Lasek et al., 83) and that this stabilizing influence may not be fully in force until late in development after the final pattern of synaptic connections has been established (Carden et al.,87; Shaw & Weber, 82). Using immunohistochemistry, we have studied the morphological disposition of NF protein in mouse parietal cortex as the brain matures. We have used 3 monoclonal antibodies (mab) from Sternberger-Meyer, Inc. These mabs bind to the H protein or the M and H proteins of the NF triplet, and they discriminate between 2 distinct phosphorylation states of the H protein. We also used a mab against glial fibrillary acidic protein. In the adult, NF immunoreactivity occurs in white matter and in distinct layers of the cortex. In the cingulate cortex 8 layers can be counted. The layered pattern is different for antibodies that bind to different phosphorylation states. Different mabs are distinct in their affinity for the somata of pyramidal cells. Before p3 the parietal cortex shows little immunoreactivity with these mabs. The layering is not mature until well-after p14. As early as p7 bushy clusters of fibers can be seen that are probably located in the hollows of whisker barrels. (Support from the Med. Res. Found. of Ore.)

414.9

IMMMUNOCYTOCHEMICAL IDENTIFICATION OF INTERMEDIATE FILAMENT PROTEINS IN THE DEVELOPING FROG NERVOUS SYSTEM. B. G. Szaro and H. Gainer. Lab. Neurochem., NINCDS, NIH, Bethesda, MD 20892.

Intermediate filament (IF) proteins from the adult Xenopus laevis nervous system were characterized immunocytochemically and on Western blots with antibodies to mammalian IF proteins. Three of these proteins were identified as cytokeratin-like (49, 55, 58 kD), one as vimentin-like (53 kD), two as GFAP-like (60 and 67 kD), and three as neurofilament-like (NF; 73, 175 and 200 kD). These antibodies were then used to observe the expression of IF proteins in the developing Xenopus nervous system from stages 12 to 42. As early as stage 19, cytokeratin-like immunoreactivity was present in the neural tube and was restricted to its inner lining throughout early neural development. Vimentin-like immunoreactivity in radially oriented neuroepithelial cell processes appeared by stage 22 in the spinal cord and rhombencephalon, stage 29/30 in the retina, and stage 33/34 in the prosencephalon. 175 kD NF protein immunoreactivity was detected shortly after the onset of neurite outgrowth in the anterior spinal cord and rhombencephalon between stages 22 and 24, and in the retina by stage 29/30, and appeared earlier than either 73 or 200 kD NF immunoreactivity. In addition, GFAP-like immunoreactivity was found in radial cells in the neural tube by stage 24, implying that the onset of glial cell differentiation occurs relatively early in Xenopus.

414.10

THE ADULT OLFACTORY SYSTEM EXPRESSES JUVENILE FORMS OF MICROTUBULE-ASSOCIATED PROTEINS. C. Viereck*, R.P. Tucker*, and A. Matus. Friedrch Miescher Inst, Box 2543, 4002 Basel, Switzerland.

Using monoclonal tau, MAP2 and MAP5 antibodies and immunoblotting, we demonstrate that the adult olfactory bulb, unlike the adult cerebellum and cerebral cortex, expresses the juvenile forms of these cytoskeletal components. Furthermore, we show that, in the juvenile brain and adult olfactory bulb, MAP5 resolves into two distinct bands which we refer to as MAP5a and MAP5b and that MAP5a is a highly phosphorylated form of MAP5. In the adult cerebellum and cerebral cortex, both the level and abundance of MAP5a relative to MAP5b are lower than in the juvenile brain and adult olfactory bulb. Immunohistochemistry shows that anti-tau stains only a subset of the axonal bundles in the olfactory nerve as well as dendrite-like processes in the sensory epithelium. Anti-MAP2 stains dendrites in the olfactory bulb, and anti-MAP5 staining is most intense in the olfactory nerve. The continued expression of juvenile tau, MAP2 and MAP5 forms in the adult CNS, where neurite outgrowth is still taking place, suggests a role for these proteins in the regulation of neurite outgrowth.

414.11

AUTORADIOGRAPHIC ANALYSIS OF CHICK RETINAL DEVELOPMENT IN CULTURE VERSUS IN OVO. S.G. Spence* and J.A. Robson. Department of Anatomy and Cell Biology. S.U.N.Y. Health Science Center, Syracuse, NY 13210.

We have compared patterns of ^{3}H-thymidine incorporation during neurogenesis in vitro and in ovo. In ovo injections were made in embryonic day 6-10 chicks (E6-E10). At E12 the embryos were fixed and the posterior retina from each eye was embedded in epon, sectioned and processed for autoradiography. In parallel experiments, pieces of posterior retina from E6 embryos were cultured in the presence of ^{3}H-thymidine. Label was added to the medium for 24 hours on day 1 in culture (E6+0) or for 3 hours on day 2, 3 or 4 in culture (E6+1, +2 or +3). Retinas were fixed at E6+4 and processed for autoradiography as above. Results show that the patterns of ^{3}H-thymidine incorporation in culture are nearly identical to those in ovo. Ganglion cells and photoreceptors are labeled in E6+0 explants and E6 embryos while cells in the inner nuclear layer (INL) are labeled in older specimens. In E6+3 cultures most labeled cells are restricted to the outer half of the INL. The most obvious difference between retinas is a reduction in the number of ganglion cells in the explants. However, our results differ from those of Kahn (Dev. Bio. 38: 30, 1974) who reported that most ganglion cells undergo their final divisions between E3 and E5. Our results indicate that this occurs between E6 and E7. (Supported by NIH Grant EY-03940 and NATO Collaborative Research Grant 0235/87).

414.12

RETINAL DIFFERENTIATION IN THE SEA LAMPREY, PETROMYZON MARINUS, WITH A TIME-SCALE IN YEARS. K. Rubinson and H. Cain.* Dept. Physiol. and Biophysics, NYU Med. Ctr., New York, NY 10016.

The retina of the larval lamprey consists of a mature central retina and a relatively undifferentiated peripheral zone . The histological and functional differentiation of the peripheral zone is completed during metamorphosis (Rubinson, et al., '77; Rubinson and Cain, '83). Relating the premetamorphic steps in retinal differentiation to the size of the animal and, thereby to its age, indicates that differentiation of the lamprey retina spans at least five years and may not be a continuous process.

Specimens with a histologically undifferentiated peripheral retina ranged in length up to 77mm (n=5, mean=64.2). Specimens in which neuroblastic and ganglion cells are the only distinguishable peripheral elements ranged from 68 to 90mm in length (n=4, mean=77.3). The differentiation of other cells was seen in lamprey of 102 to 134mm (n=6, mean=114). Photoreceptor inner and outer segments were only seen in animals entering metamorphosis.

Thus, ganglion cells begin to appear when the lamprey are 1 to 2 years old and differentiation of the remaining neuroblasts requires a minimum of 2 additional years. Beyond 4 years of age, the mean length of unmetamorphosed lamprey does not change. As the age at metamorphosis ranges from 5 to as much as 15 years, it seems that the completion of the retina can be deferred for a number of years.

414.13

GROWTH OF THE VOMERONASAL ORGAN IN GARTER SNAKES. D. A. Holtzman* (SPON: M. Halpern). Program in Neural and Behavioral Sci., SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

Changes in cellular composition and in volume of the vomeronasal organs (VNO) of embryonic and neonatal garter snakes were examined. Microprojection drawings of 10 micron serial sections were used to calculate the volumes of the supporting cell (SC) and bipolar cell (BP) layers in VNO's. The SC layer decreases to a quarter of its volume from mid-gestation to the period just before birth. During the same period of time, the BP layer increases to 4 times its volume. At birth, the volume of the SC layer has not changed, but the volume of the BP layer has increased by 2.5 times. Experiments using tritiated thymidine autoradiography suggest a differential generation of cells in the SC and BP layers of the VNO.

Supported by NIH grant NS 11713.

414.14

DOES THE MOUSE BRAIN HAVE ITS OWN MACROPHAGES? Hao, C.,* Richardson, A.,* and Fedoroff, S. Department of Anatomy, University of Saskatchewan, Saskatoon, SK. Canada.

When astroglia cultures are subjected to unfavorable growth conditions, large numbers of macrophages appear. These cells are ameboid, highly phagocytic, and excrete lysozyme. In established astroglia cultures initiated from mouse embryos at Theiler stages T14(E9), T18(E11), T21(E13), T23(E15), T25(E17) and from newborn animals, when grown for an additional 10 days without medium change, macrophages were present and the medium contained lysozyme. In contrast, cultures that were fed had no, or only a few macrophages and the medium contained no, or very low concentrations of lysozyme.

Macrophages in neglected astroglia cultures were compared with macrophages from adult mouse spleen and peritoneal cavity on the basis of their growth characteristics, response to trophic factors, presence of α-naphthyl butyrate esterase, peroxidase, Mac-1, Mac-3, M1/70, Ia, DiI-ac-LDL, GFAP and vimentin. We conclude that macrophages in the neglected astroglia cultures share many macrophage-specific characteristics with spleen and peritoneal cavity macrophages, but that some notable differences exist, indicating the presence of a specific type of macrophage in mouse brain. Precursors of these macrophages are present in very early embryonic stages of brain development. This work was supported by MRC of Canada Grant MT4325.

416

SYMPOSIUM: DEVELOPMENT OF FUNCTIONAL HETEROGENEITY AMONG SENSORY NEURONS. S.A. Scott, SUNY Stony Brook (Chairperson), L.M. Mendell, SUNY Stony Brook, A.W. Mudge*, Univ. Coll. London, A.M. Davies, St. George's Hosp. Med. Sch., J. Dodd, Columbia Univ. CPS, E. Frank, Univ. Pittsburgh Med. Sch.

Dorsal root ganglia (DRG) contain a diversity of sensory neurons. This symposium will consider some of the characteristics that distinguish subsets of sensory neurons, and how these differences arise during embryonic development. Dr. Scott will introduce the symposium by providing a brief overview of sensory neuron heterogeneity. Dr. Mendell will present recent findings on the physiological and anatomical specialization of individual sensory neurons as correlated with peripheral receptor type. Dr. Mudge will describe the early commitment of a subpopulation of DRG neurons to the Substance P phenotype, and the role of Schwann cells in determining DRG neuron morphology. Dr. Davies will consider the specificity and cooperation of different neurotrophic factors in regulating the survival of developing sensory neurons. Dr. Dodd will describe the surface properties of functionally distinct spinal sensory neurons, and will discuss axon guidance in spinal sensory neuron development. Dr. Frank will describe the phenotypic specification of sensory neurons as revealed by their spinal projections.

417

CODING AND EXECUTION OF MOVEMENT IN THREE DIMENSIONS. B.Cohen, V.Henn, T.Raphan, A.Georgopoulos, J.Soechting, J.Hollerbach.

Previous studies of coding in motor systems have often, by necessity, been limited to analysis of movement in one or two dimensions. Nevertheless, we live in a three dimensional world embedded in a gravitational environment, and the brain has evolved to code and produce motion in this environment. The goal of this symposium is to give insight into how movement in three dimensions is coded and executed by the oculomotor and skeletal motor systems. VOLKER HENN will consider whether the mechanism that produces saccadic eye movements is organized around motion in planes of space parallel to the planes of the semicircular canals or the eye muscles. THEODORE RAPHAN will present data and a model relating the mathematical structure of the dynamical system representing the slow component of the VOR to the spatial coordinate basis determined by gravity. APOSTOLOS GEORGOPOULOS will give evidence that coding of limb movement in neurons in the motor cortex is, on average, three dimensional. JOHN SOECHTING will consider representation of sensory-motor maps, obtained from psychophysical data from humans. JOHN HOLLERBACH will consider the interface between motor performance and robotics as revealed through study of kinematic calibration. The symposium will show the importance of mathematical modelling and experimental verification in generating a unified approach to understanding and predicting the behavior of sensory motor systems in three dimensions. The experimental and modelling approaches should be of interest to physiologists working on the oculomotor and skeletal motor systems as well as to those interested in designing robots that move and perform visual-motor tasks.

HUMAN BEHAVIORAL NEUROBIOLOGY IV

418.1

DEVELOPMENT IN HUMAN INFANTS OF VISUAL RECOGNITION MEMORY. W. Overman, J. Bachevalier , P. Roland*, M. Turner*, Dept. Psychol., U.N.C.-Wilmington, Wilmington, N.C. 28401 and Lab. Neuropsychol., NIMH, Bethesda, MD 20892

Two trial-unique visual recognition memory tasks, delayed non-match or match-to-sample (DNMS or DMS), were used to assess visual recognition memory in human infants and adults. Nine females aged 12-32 months, 6 males aged 14-26 months, and 12 adults (6 females and 6 males, 19-55 years of age) were trained without verbal instructions on either DNMS or DMS tasks in a WGTA. Subjects were tested daily to a criterion of 13/15 correct responses for two consecutive days. Their recognition memory was further challenged by increasing progressively the delay intervals and the lists of objects to be remembered. For DNMS there were significant inverse correlations between age and trials to criterion in both infant females ($r=-.94$) and infant males ($r=-.83$). On DNMS females reached adult levels of performance (2.5 days and 3 errors to criterion) around 20 months of age and males did so at around 26 months of age, suggesting that at these respective ages maturational events occur that enable adult performance on non-match tasks. In contrast, on DMS adult performance was much more delayed in both infant females and males, indicating a slower maturation of behavarioral inhibition, presumably from the prefrontal cortex.

418.2

RIGHT TEMPORAL-LOBE CONTRIBUTION TO GLOBAL VISUAL PROCESSING. J. Doyon* and B. Milner. Montreal Neurological Institute, McGill University, Montreal, Canada, H3A 2B4.

The contribution of the right temporal lobe to global visual processing was investigated in 36 patients with unilateral temporal- or frontal-lobe excisions and 15 normal control subjects. The experiment used hierarchically structured stimuli, each consisting of one large abstract design (global level) created by the spatial arrangement of identical smaller abstract designs (local level). The subjects were tested under two experimental conditions (global, local) of a Stroop-type, reaction-time task. In the local condition, the subjects were asked to focus their attention on the small designs and to ignore the large design, whereas they were instructed to do the reverse in the global condition. The results showed that, in the local condition, the right temporal-lobe group was less affected than other groups by interference from the overall configuration of the stimulus. As predicted, this reduced sensitivity to the global aspect of the stimuli was unrelated to the extent of hippocampal removal. These findings support the hypothesis that the human right temporal neocortex contributes to global visual processing.

418.3

THE ROLE OF THE FRONTAL LOBES IN THE ENCODING AND RECALL OF KINESTHETIC INFORMATION.
G. Leonard* and B. Milner (SPON: M. Lassonde). Montreal Neurol. Inst., McGill Univ., Montreal, Canada H3A 2B4.

One hundred and fifty two patients with unilateral temporal- or frontal-lobe excisions and 41 normal control subjects were tested on four kinesthetic recall tasks. The first two studies required subjects to monitor peripheral feedback, in order to duplicate either the distance or the end-position of examiner-defined arm movements. In the next two tasks, the subjects selected the movements to be recalled, and hence reliance on feedback was reduced. Temporal lobectomy did not interfere with performance, except on the examiner-defined kinesthetic location task where large left or large right hippocampal resection produced an impairment following 30 s of counting. Patients with left frontal-lobe or small right frontal-lobe excisions performed all tests normally, but those with large right frontal-lobe removals were impaired on the examiner-defined tasks across all delay conditions, and with both hands. The results point to an important role played by the right frontal lobe in the monitoring of kinesthetic feedback both during the presentation of the movements and during the recall attempt.

418.4

AMNESIA FOLLOWING MEDIAL TEMPORAL LOBE DAMAGE IN MONKEYS: THE IMPORTANCE OF THE HIPPOCAMPUS AND ADJACENT CORTICAL REGIONS. S. Zola-Morgan, L.R. Squire, and D.G. Amaral. V.A. Medical Center, San Diego, CA 92161, Dept. of Psychiatry, U,C.S.D., La Jolla, CA 92093, and the Salk Institute, San Diego, CA 92138.

Monkeys with bilateral medial temporal lobe lesions that damage the hippocampal formation, amygdala, perirhinal cortex, and parahippocampal cortex (H^+A^+ lesion) are severely impaired on tasks such as delayed nonmatching to sample (DNMS), which are sensitive to human amnesia. A more restricted lesion of the hippocampal formation that includes much of the parahippocampal cortex (H^+ lesion) produces a significant but less severe deficit. To determine what damage in the larger H^+A^+ lesion accounts for the more severe memory impairment, we prepared three groups of operated animals. Animals with stereotaxic lesions limited to the amygdala demonstrated no detectable deficit on DNMS. Moreover, the addition of a selective amygdaloid lesion to the H^+ lesion did not increase the impairment associated with the H^+ lesion. However, when both the perirhinal and parahippocampal cortices were damaged bilaterally without direct involvement of the amygdala or hippocampal formation, animals were at least as severely impaired on DNMS as those with H^+A^+ lesions. Since the perirhinal and parahippocampal cortices provide the major input to the monkey hippocampal formation, these data emphasize the importance of the hippocampal system in temporal lobe amnesia.

418.5

INDEPENDENCE OF MEMORY FUNCTIONS AND EMOTIONAL BEHAVIOR: SEPARATE CONTRIBUTIONS OF THE HIPPOCAMPAL FORMATION AND THE AMYGDALA. P. Alvarez-Royo*, M. Meseches*, J. Allen*, W. Saltzmann*, L.R. Squire and S. Zola-Morgan (SPON: S.A. Hillyard). Group in Neurosciences and Dept. of Psychiatry, U.C.S.D., La Jolla, CA 92093, and V. A. Med. Ctr., San Diego, CA 92161.

Monkeys with large medial temporal lobe lesions, including the hippocampus, amygdala and surrounding cortical areas (H^+A^+ lesion), are impaired on tasks sensitive to human amnesia. However, damage to the areas included in the H^+A^+ lesion can also cause changes in emotional behavior (e.g. the Kluver-Bucy syndrome). One possibility is that memory might be affected by these changes in emotional behavior.To examine this possibility, we compared the performance of monkeys with extensive or partial lesions of the medial temporal lobe on two behavioral batteries. The first measured performance on four different memory tasks. The second measured emotional behavior by evaluating responses to a variety of inanimate objects (e.g. a model of a snake, a black rubber boot, M&M candy).

Monkeys with H^+A^+ lesions were impaired on the memory tasks, and also showed abnormally elevated emotional reactivity. Monkeys with selective and circumscribed amygdala lesions performed the memory tasks normally but showed elevated emotional reactivity. Monkeys with damage to components of the hippocampal formation but without amygdala damage were impaired on the memory tasks but showed normal emotional reactivity. This double dissociation shows that memory impairment is independent of the observed changes in emotional behavior. The implications of these findings for the function of the hippocampal formation and the amygdala will be discussed.

418.6

THE NEUROLOGY OF MEMORY: QUANTITATIVE ASSESSMENT OF RETROGRADE AMNESIA IN TWO GROUPS OF AMNESIC PATIENTS. L.R. Squire, F. Haist, A.P.Shimamura, VA Medical Center, Dept. of Psychiatry, Univ. of California, San Diego, CA

Using six tests of remote memory, we evaluated the extent, severity, and stability of retrograde amnesia in two groups of amnesic patients--seven patients with alcoholic Korsakoff's syndrome and five patients with amnesia due to an anoxic or ischemic episode. The severity and extent of retrograde amnesia was similar for the two groups and was temporally graded across a period of about 15 years. The results support the following conclusions: 1) extensive, temporally-graded retrograde amnesia, which has been observed frequently in patients with Korsakoff's syndrome, occurs rapidly in other amnesic patients as well, even when their memory impairment appears well circumscribed; 2) patients with presumed damage to either the medial temporal or the diencephalic brain structures linked to memory functions can produce a similar kind of retrograde amnesia; 3) the impairment was stable across testing occasions, and thus does not reflect difficulty accessing an intact memory store that can be overcome with sufficient retrieval opportunities; 4) very remote memory, at least for factual information, can be intact in amnesia; 5) the structures damaged in amnesia support memory storage, retrieval, or both during a lengthy period of reorganization, after which representations in memory can become independent of these structures.

418.7

TRANSIENT GLOBAL AMNESIA: CHARACTERIZATION OF RETROGRADE AMNESIA IN SIX PATIENTS: M. Kritchevsky* and L.R. Squire. (SPON: N. Butters), V.A. Medical Center, San Diego, CA, 92161, Depts. Neurosciences and Psychiatry, UCSD, La Jolla, CA.

Transient global amnesia (TGA) is a short-lasting neurological condition in which memory impairment is the prominent deficit. We studied six patients in 1987, during and after their episode. During TGA, all patients were impaired on tests of new learning ability for verbal and nonverbal material. Additionally, during the episode, patients had difficulty recalling news events from the time period 1960-1985. Recall for events from 1950-59 was similar during and after TGA. During TGA, recognition memory for news events was impaired for the time period 1980-1985 but not for more remote time periods. Memory was normal one month after TGA. Results from a personalized test of past memory given during TGA similarly suggested that retrograde amnesia, albeit patchy and variable, affected some public and personal memories which had occurred as long ago as 1960. The memory impairment exhibited by these patients will be compared to the memory impairment exhibited by other patients with chronic amnesia.

418.8

MEMORY FOR TEMPORAL ORDER IN PATIENTS WITH FRONTAL LOBE LESIONS AND PATIENTS WITH AMNESIA. A.P. Shimamura*, J.S. Janowsky*, and L.R. Squire (SPON: H. Neville). Dept. of Psychiatry, Univ. of Calif., San Diego, La Jolla, CA 92093.

Patients with frontal lobe lesions, patients with Korsakoff's syndrome, patients with amnesia due to an anoxic or ischemic episode, and control subjects were asked to remember the order in which 15 words were presented. Memory for sequential (i.e., temporal) order was assessed by correlating a subject's ordering of the words with the actual presentation order. Patients with frontal lobe lesions exhibited a deficit in temporal order memory, despite normal recognition memory for the words. Amnesic patients exhibited both impaired temporal order and word recognition memory. Importantly, patients with Korsakoff's syndrome were more impaired on the temporal order test than anxoic-ischemic patients, consistent with their known frontal lobe atrophy. Similar results were obtained in a second experiment in which subjects were asked to place in chronologoical order 15 public events that occurred between 1941 and 1985. The findings demonstrate that frontal lobe lesions particularly affects temporal order memory, whereas damage to the areas that cause amnesia (i.e., diencephalic and medial temporal areas) affects both item memory and temporal order memory.

418.9

SOURCE AMNESIA IN PATIENTS WITH FRONTAL LOBE LESIONS AND THE ELDERLY. J.S. Janowsky,* A.P. Shimamura,* and L.R. Squire. (SPON: T. Jernigan). VA Medical Center, San Diego, CA 92161 and Dept. of Psychiatry, U.C.S.D., La Jolla, CA 93093.

Information is often recalled in conjunction with its source (i.e. when or where the information was learned). Previous studies showed that some amnesic patients exhibit source amnesia: they can recall a few facts taught in a recent session but they attribute the information to some other context. This study investigated the ability of patients with frontal lobe lesions and two groups of control subjects (elderly and young) to learn new facts as well as to recall the source of the information. In two experiments, patients with frontal lobe lesions recalled facts as well as the control subjects. However, both patients with frontal lobe lesions and elderly control subjects were more likely than young subjects to attribute facts learned within a test session to an incorrect source. In addition, patients with frontal lobe lesions but not the elderly or young control subjects sometimes attributed facts to the test session itself, when these facts had actually been known to the subject prior to the test session. This study shows that source errors can occur in patients with frontal lobe lesions, as well as in healthy elderly subjects, in the absence of amnesia. The frontal lobes may play a special role in helping join information in memory to the context in which it was learned.

418.10

Amnesics' Relatively Preserved Recognition in Implicit Memory.

William Hirst,* Elizabeth A. Phelps,* Marcia K. Johnson* and Bruce T. Volpe. (SPON: F. McDowell). Graduate Faculty, New School for Social Research, NY, NY, Princeton University, Cornell University Medical College.

Several theorists have divided the memory system into a component responsible for the explicit retrieval of declarative memories and a component responsible for the implicit retrieval of procedural memories (Squire, 1986). This dichotomy is supported in part by finding that human anterograde amnesia disrupts explicit declarative memory while leaving implicit procedural memory intact. Recently, it has been reported that amnesic recognition is relatively preserved when compared with amnesic recall. In as much as recognition is an explicit declarative memory task this result could suggest that aspects of amnesic explicit memory remain intact. However, amnesics' unexpectedly good recognition could build solely on their intact implicit memory. Three experiments test this hypothesis. In two, amnesic recognition is raised to normal levels even in the absence of any signs of priming as measured by stem completion and perceptual identification. In the third, factors affecting amnesic recognition such as depth of processing are shown to have no effect on amnesic priming suggesting that amnesic recognition and priming involve different mechanisms. A model accounting for the results is discussed.

418.11

VOLUNTARY CONTROL OF ATTENTION IS IMPAIRED IN KORSAKOFF' SYNDROME. RG Mair, DB Flint,* U New Hampshire and Research Service 151C, VAMC, Brockton, MA 02401, A. Inhoff*, SUNY Binghamton, PJ Langlais, San Diego VAMC.

We compared the performace of 9 Korsakoff (K) and 9 alcoholic control (AC) subjects on a series of chronometric tasks using CRT presentation and a manual response board. Tasks included reaction times (rt) facilitated by stimulus driven, subject driven, and voluntarily controlled shifts in attention; as well as Sternberg-type short term memory retrieval and sequential finger tapping. Korsakoffs exhibited slower rt's in all tasks. Although the K's showed normal superiority of rt's to valid as opposed to invalid cues, they were significantly slower on subject driven than stimulus driven attentional tasks, a pattern opposite that observed among controls. While the AC's were able to utilize cues in the voluntarily driven attentional task at a delay of 250 msec, K's showed no such benefit until a delay of 1000 msec. On both the memory retrieval and motor sequencing tasks, K's were consistently slower for all response conditions. The speed of retrieval of items from short term memory correlated significantly with performance on the attentional tasks.

418.12

PROCEDURAL LEARNING IN SCHIZOPHRENIA: EVIDENCE FROM TOWER OF HANOI-LIKE TASKS. T. E. Goldberg*, J. A. Saint-Cyr and D. R. Weinberger. Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032.

Neuropsychological paradigms have indicated the presence of frontal lobe dysfunction in schizophrenia. However, the matter of task difficulty has not been adequately addressed; that is, patients might do worse on these tasks simply because they are harder. Also, prefrontal cognitive failure might reflect "downstream" dysfunction in the basal ganglia. We attempted to examine these issues by using variants of the Tower of Hanoi puzzle. A three disk version is thought to emphasize problem solving and has been found to be sensitive to frontal lobe lesions, while a more difficult four disk version is thought to emphasize procedural learning and is sensitive to basal ganglia disease. Relative to controls, schizophrenic patients performed as poorly on the easier three disk version as they did on the more difficult four disk version. Moreover, in repeated trials, patients were able to learn the four disk tower at a rate similar to that of normal subjects, though they learned the three disk Tower much more slowly. These results suggest that poor performance on frontal lobe tests is not simply a function of task difficulty and that certain aspects of basal ganglia function may be relatively more intact in schizophrenia than frontal lobe function.

ION CHANNELS: LIGAND-GATED II

419.1

LABELING OF TORPEDO NICOTINIC ACETYLCHOLINE RECEPTOR (AChR) WITH THE HYDROPHOBIC PHOTOREAGENT 3-(TRIFLUOROMETHYL)-3-(m-[^{125}I]IODOPHENYL)DIAZIRINE ([^{125}I]TID). **B.H. White* and J.B. Cohen (SPON: D. Frail). Dept. of Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.**

We have studied the photoincorporation of the hydrophobic probe [^{125}I]TID into the AChR of T. Californica postsynaptic membranes. [^{125}I]TID labels all four subunits of the AChR. Two regions of the α-subunit incorporate label as determined by digestion with S. aureus V8 protease: the first, defined by a 20 kDa cleavage fragment, begins at residue Ser-173 and contains the hydrophobic segments M1-M3; the second extends from Asn-339 through the hydrophobic segment M4. Two fragments, spanning most of the amino terminal region from Ser-1 to Glu-161, fail to incorporate [^{125}I]TID.

[^{125}I]TID labeling of all AChR subunits is strongly inhibited (75-95%) by the agonist carbamylcholine (100 μM), an effect that is blocked by the competitive antagonist α-bungarotoxin and is restricted, on the α-subunit, to the 20 kDa V8 fragment. The noncompetitive antagonist histrionicotoxin (30 μM) also strongly inhibits AChR labeling, but 50 μM phencyclidine does not. Non-radioactive TID also specifically inhibits [^{125}I]TID labeling of the AChR (IC_{50}=10 μM). We conclude that [^{125}I]TID acts both as a nonspecific label of AChR regions exposed to lipid, and as an affinity label for a site (or sites) on the AChR distinct from, but coupled to, the agonist and noncompetitive antagonist binding sites. (Supported in part by USPHS NS 19522 and 22828 and a NSF predoctoral fellowship to B.W.)

419.2

EXPRESSION OF AVIAN GENES ENCODING NEURONAL NICOTINIC ACh RECEPTORS. E.Cooper, C.R.Bader, D.Bertrand, D.Rungger, P.Nef, S.Couturier and M.Ballivet. *Dept of Physiol. McGill Univ. Montreal,P.Q. H3G 1Y6 and Dpt de Physiol. CMU, Dpt de Zool. and Dpt de Bioch., Université de Genève. CH-1211 Genève 4, Switzerland.*

Recently, a family of genes encoding proteins related to muscle endplate nAChR subunits has been isolated from the avian genome and sequenced (Nef et al., *EMBO J.*, 7:595,1988).These genes (and their protein products) have been termed α 2, α 3, α4 and nα (non-α) and are expressed in the central and peripheral nervous system. All neuronal alpha subunits possess the pair of vicinal cysteine residues thought to be part of the ACh binding site, whereas these cysteines are lacking in the nα subunit. In this study, we used voltage clamp and patch clamp techniques to measure the physiological properties of nicotinic ACh receptors expressed by oocytes 1-5 days after nuclear injection with cDNA for α4 and nα linked to a heat-shock promoter. The DNA injection technique was used because we found that it gave more reliable ACh receptor expression compared to oocytes injections with cRNA. Heat-treated oocytes injected with both α4 and n-α constructs expressed up to 2-3uA of ACh-induced currents that could be reversibly blocked by hexamethonium but that were insensitive to α-bungarotoxin. From dose-response experiments, we determined that the slope of log-response vs log-concentration plots up to 80nM ACh was 1.5, suggesting that the functional receptors assembled with at least two α4 subunits. Single channel measurements of receptors from outside-out patches demonstrated that these receptors had linear I-V curves, reversal potentials of about -7mV and single-channel conductances of 20pS. Interestingly, the receptors, which were stable in cell-attached patches, gradually disappeared from outside-out patches over few minutes; this suggests that intracellular factors may modulate the function of these neuronal nicotinic receptors.

419.3

DIFFERENCES IN THE PHOSPHORYLATION OF UNASSEMBLED, ASSEMBLED BUT CYTOPLASMIC, AND SURFACE ACETYLCHOLINE RECEPTORS. W.N. Green* and T. Claudio. (SPON: R. Wyman) Dept. of Cell. and Molec. Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510

The *in vivo* phosphorylation of *Torpedo* nicotinic acetylcholine receptor (AChR) subunits was studied using a mouse fibroblast cell line in which the four *Torpedo* AChR subunit cDNAs had been stably integrated into the host cell genome (Claudio, et al., *Science* 238:1688, 1987). Each of the subunits is synthesized, glycosylated, assembled into AChR complexes, and the complexes are expressed on the cell surface where they display all of their proper pharmacological and physiological properties. Intact AChR-fibroblast cell lines were first incubated with ^{32}P and unlabeled α-bungarotoxin (BuTx), then solubilized, followed by sequential immunoprecipitations with BuTx-specific or subunit-specific antisera. These differential immunoprecipitations resulted in the isolation of three classes of AChR subunits: 1) unassembled, cytoplasmic subunits, 2) assembled, cytoplasmic AChR complexes, and 3) assembled, cell surface AChR complexes. The degree and pattern of AChR phosphorylation differed among these three AChR pools. Phosphorylation of the γ and δ subunits was observed in the unassembled subunits and the surface AChR complexes, whereas only the δ subunit (and possibly β) was phosphorylated in the cytoplasmic AChR complexes. No phosphorylation of the α subunit was detected in any of the AChR pools. The cAMP-dependent protein kinase stimulators forskolin, cAMP-S, and cholera toxin, increased phosphorylation of the unassembled and cell surface AChR pools with no corresponding increase in the phosphorylation of the cytoplasmic assembled AChR pool. Further analysis of the role phosphorylation may play in AChR assembly and/or function is continuing.

419.4

TEMPERATURE-SENSITIVE EXPRESSION OF ALL-*TORPEDO* AND *TORPEDO*-RAT HYBRID ACHRS IN MAMMALIAN CELLS. H.L. Paulson* and T. Claudio. (SPON: E. Manuelidis) Dept. of Cell. and Molec. Physiology, Yale Univ. Sch. of Medicine, New Haven, CT 06510

We have recently shown that mouse fibroblasts express fully functional, cell surface *Torpedo californica* nicotinic acetylcholine receptors (AChRs) after the four subunit cDNAs have been stably integrated into the host cell genome (Claudio, et al., *Science* 238:1688, 1987). Although each of the subunit polypeptides is synthesized at 37°C (normal mammalian cell growth conditions), functional AChR complexes are only expressed at temperatures lower than 37°C. The four *Torpedo* AChR subunit cDNAs have also been stably integrated into the genome of a rat muscle L6 cell. Again, we find that expression of *Torpedo* AChRs only occurs at temperatures lower than 37°C whereas expression of endogenous rat AChRs occurs at both 37°C and lower temperatures. These results demonstrate that the temperature-sensitive phenomenom is not fibroblast-specific, however it does appear to be *Torpedo*-specific. Another stable L6 muscle cell line was established in which only the *Torpedo* α subunit cDNA was integrated (L6-α). Expression of *Torpedo*-rat hybrids in L6-α cells only occurred at temperatures lower than 37°C and in addition, three classes of AChRs could be isolated. AChRs were expressed that contained two rat, two *Torpedo*, or one rat and one *Torpedo* α subunit, demonstrating that the two α subunits in an AChR complex need not originate from the same polysome.

Further analysis of the *Torpedo* temperature-sensitive phenomenom suggests that the temperature-sensitive step occurs before assembly and that it probably involves an altered polypeptide conformation. 1) Once hybrid or all-*Torpedo* AChRs are formed, they are stable at 37°C. 2) *Torpedo* α subunits show a 2- to 3-fold increase in binding to α-bungarotoxin upon shift from 37°C to 26°C.

419.5

ISOLATION OF A SECOND MUSCLE ACHR ALPHA-LIKE SUBUNIT FROM *XENOPUS LAEVIS*. D.S. Hartman* and T. Claudio. Dept. of Cell. and Molec. Physiology, Yale Univ. Sch. of Med., New Haven CT 06510.

Cloning of the muscle nicotinic acetylcholine receptor (AChR) α, γ, and δ subunit cDNAs from *Xenopus laevis* stage 17 or 22 cDNA libraries has been reported (Baldwin, T. et al., *J. Cell Biol*, 106:469, 1988). We report here, the isolation of a fourth putative AChR subunit (*Xenopus* muscle α2, xmα2) from a stage 17 embryonic *Xenopus* cDNA library. We have tentatively assigned xmα2 as an α subunit for the following reasons. Expression in a rabbit reticulocyte lysate system of *in vitro* transcribed xmα2 mRNA makes a 40 kd polypeptide that is immunoprecipitated by polyclonal antisera directed against *Torpedo* α subunit. The deduced amino acid sequence of clone xmα2 revealed that it is composed of 437 amino acids (found in all muscle-like α subunits), it contains conserved cysteine residues corresponding to amino acid residues #128, 142, 192, 193 (*Torpedo* α numbering), and it contains the one potential N-linked glycosylation site corresponding to *Torpedo* residue #141. A comparison of the amino acid similarities (identity plus conservative substitutions) among xmα2, *Torpedo* α, and the Baldwin et al. *Xenopus* α clone (xα), revealed: ~80% similarity beween xmα2 or xα and *Torpedo* α; <50% similarity between xmα2 or xα and *Torpedo* β; ~80% similarity beween xmα2 and xα. The xmα2 and xα clones show similarities throughout their entire lengths with the most divergence occuring between the amino terminus and the first putative transmembrane domain, M1. Transcripts from both xmα2 and xα are found in adult *Xenopus* muscle and appear to be coordinately expressed throughout early embryonic development (oocyte, stages 10, 17, 22, 25, 30, 35).

419.6

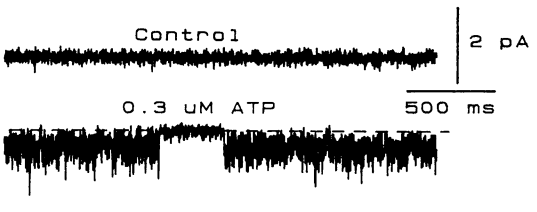
ACTIVATION AND BLOCK OF SINGLE NICOTINIC RECEPTOR CHANNELS OF THE FROG ENDPLATE BY DECAMETHONIUM. C.G. Marshall, D.C. Ogden* and D. Colquhoun*. Dept. of Pharmacology, University College, London WC1E 6BT, U.K.

Agonists which produce a small maximal effect are classically termed "partial agonists", and decamethonium (DECA) acts as such at the nicotinic receptor. Adams and Sakmann (Proc. Natl. Acad. Sci. USA (1978) 75 2994-2998) suggested that this effect derived simply from open channel block by DECA, in contrast to the view that partial agonists are inherently ineffective at receptor activation. We have used single channel recording to obtain data for DECA as an agonist and channel blocker over a wide range of concentrations (5-500μM), and data were interptreted in terms of a sequential reaction scheme. A component of the shut time distribution of mean duration 1.75-2.84 ms was identified as blockage gaps, and the blockage frequency plot gave a slope, k_{+B}, of 3.6 x 10^7 $M^{-1}s^{-1}$, intercepting the origin as expected. At 5μM DECA, the mean open time was 452μs, and a plot of reciprocal mean open time gave a slope, k_{+B}, of 3.0 x 10^7 $M^{-1}s^{-1}$, and an intercept, α, of 2076s^{-1}. We estimate the equilibrium dissociation constant for open channel block, K_B, to be 7-15μM. Our data is consistent with a simple open channel block. The maximum value of P_{open} is less than 5%, and although DECA is clearly a potent blocker, we cannot yet discriminate between a small maximal effect purely due to channel block, and a low intrinsic efficacy.

419.7

ATP-ACTIVATED CHANNELS IN RAT AND FROG SENSORY NEURONS. **B.P. Bean, C. A. Williams*, and P. W. Ceelen*. Department of Neurobiology, Harvard Medical School, Boston, MA 02115**

External ATP (Kd~3 uM) induced an increase in conductance in about 30% of bullfrog and 20% of rat DRG neurons tested. As reported by Krishtal et. al. (Neurosci. Lett. 35:41,1983), the ATP-induced current was cation-selective, inwardly-rectifying, and reversed near 0 mV. The induced current increased 3.5-4 fold for a doubling in [ATP], suggesting 2:1 binding. The rapid onset of ATP-activated current (τ ~ 300 ms at 0.3 uM ATP, τ < 15 ms at 100 uM) is nearly diffusion-limited, so that second messenger mediation is unlikely (or extraordinarily rapid). Single channels in outside-out patches flickered rapidly (τ ~ 10 usec) when activated, with a mean current of about 0.5 pA at -100 mV. The fraction of time a channel was in the activated, flickery state approached 1 at high [ATP]. Whole-cell noise showed exactly the properties expected if such channels underly the macroscopic current.



419.8

BIOPHYSICAL AND PHARMACOLOGICAL PROPERTIES OF $GABA_A$ RECEPTOR SUBUNIT CLONES EXPRESSED IN OOCYTES E.S. Levitan, L.A.C. Blair, V.E. Dionne* and E.A. Barnard*. MRC Molecular Neurobiology Unit, Cambridge, England.

The recent cloning of the bovine brain $GABA_A$ receptor α1 and β subunits (Schofield et al., 1987) has led to the further isolation of two additional α subunit RNAs (α2, α3)(Levitan et al., submitted). The properties of these clones have been determined and compared by injecting synthetic RNA into Xenopus oocytes and studying the induced GABA-sensitive currents with voltage- and patch-clamp methods. Expressing any α-β pair of subunits together produces GABA-activated Cl^- currents that are potentiated by the pentobarbital and inhibited by bicuculline and by picrotoxin. The Cl^- channels display characteristic multiple single-channel conductance states and voltage-dependent gating. A functional difference occurs when the α subunits are interchanged: the apparent sensitivity of the receptor for GABA is shifted 30-fold from 1.3 uM to 42 uM (EC_{50}). The order of sensitivity is α2,α1,α3, with α2 being most sensitive. The results suggest that functionally distinct subtypes of the $GABA_A$ receptor exist in the brain. However, these receptors are partially impaired since they fail to show the expected sensitivity to benzodiazepines and are activated by GABA with a Hill coefficient of only 1. This suggests that additional subunits or processing is required for normal $GABA_A$ receptor function. Supported by NSF (ESL) and NIH (LACB, VED).

419.9

SINGLE SUBUNITS OF THE GABAa RECEPTOR FORM ION CHANNELS WITH PROPERTIES CHARACTERISTIC OF THE NATIVE RECEPTOR.
L.A.C. Blair, E.S. Levitan, V.E. Dionne* and E.A. Barnard*
MRC Molecular Neurobiology Unit, Cambridge, England.

Alpha and beta subunits of the GABAa receptor were expressed individually in Xenopus oocytes using RNA synthesized from cloned DNAs. GABA-sensitive, chloride-selective channels were detected several days after injection with any one of three different RNAs (α_1,α_2,α_3) or with β RNA. The channels induced by each of the single subunit RNAs were indistinguishable. They had multiple conductance levels (nominally 10, 18, 28 and 44pS), and their activity was potentiated by 20μM pentobarbital and inhibited by 10μM picrotoxin indicating that the sites for these agents are present on each subunit. Based on conservative substitutions, the α and β subunits of the GABAa receptor show ~55% amino acid sequence homology. The finding that each of α and β subunits, examined separately, form GABA-sensitive ion channels with barbituate and picrotoxin regulatory sites and normal permeation properties suggests that the amino acid sequences which confer these properties in the native receptor are within the shared domains.
Supported by NIH (LACB, VED) and NSF (ESL).

419.10

QUATERNARY AMMONIUM IONS BLOCK N-METHYL-D-ASPARTATE RECEPTOR-CHANNELS IN MAMMALIAN CENTRAL NEURONS IN CULTURE.
J. M. Wright* and L. M. Nowak. Dept. of Pharmacology, NYSCVM Cornell University, Ithaca, NY 14853.

Previous studies of N-methyl-D-aspartate (NMDA) activated channels indicated they were blocked in a voltage-dependent way by choline ions (Ascher et al., J. Physiol., 399:207,1988). We have examined the effects of two other quaternary ammonium ions commonly used to block voltage-gated K channels: tetraethyl- and tetrabutylammonium (TEA and TBA), on excitatory amino acid responses using patch clamp recording methods (whole cell and outside-out patches) from mammalian central neurons in cell culture. Kainate (20μM) and AMPA (5μM) whole cell currents were not affected by either TEA (5mM) or TBA (1mM). In contrast, TEA (1-10mM) and TBA (1mM) decreased NMDA (20μM) whole cell currents in a voltage-dependent manner with a greater effect at −60mV than at −40mV. Reduction of NMDA channel current amplitude by TEA was dose-dependent and qualitatively similar to the effects in choline. TBA appeared to cause a flickering block without a noticable change in conductance. Concentrations of Mg, Co, Ni and Mn ions, which might show similar effects, were found to be too low to account for the effects of TEA or TBA. Thus, the K-channel blockers TEA and TBA also block NMDA responses at similar concentrations as they affect K-channels. Detailed analysis of single channel kinetics will allow us to determine more about the mechanism of action of these blockers.
Supported by NIH grant NS24467.

419.11

STRYCHNINE AND GLYCINE-RECEPTOR INTERACTIONS IN CULTURED MEDULLARY NEURONS FROM EMBRYONIC RAT.
C.A. Lewis*, D.S. Faber and Z. Ahmed. Dept. Physiology, State Univ. of New York, Buffalo, NY 14214.

Characteristics of glycine-activated currents in 10 to 20 day old neurons were studied using the gigaseal whole-cell technique. Cells were voltage-clamped to -70 mV, and brief pulses of glycine were applied by pressure ejection from a second pipette containing 2 M glycine. Glycine produced inward currents that had a shift in reversal potential of +60 mV for a 10-fold reduction in external Cl^- concentration, indicating that the currents were due to activation of a Cl^- conductance.

Dose-response data were obtained for glycine in control saline and in saline containing different concentrations of strychnine (100 nM to 100 uM). Strychnine had mixed inhibitory actions on the glycine-induced currents in that both the maximum responses were decreased and the dose-response curves for glycine were shifted to the right. Glycine receptors with at least two different sensitivities to strychnine were found, based on the apparent inhibition constants (K_i). These two K_i values were associated with different Hill coefficients for the glycine responses; i.e. $K_i = 4.4 \pm 0.9 \times 10^{-8}$ M (± SEM, n=5), $n_{app} = 1.9 \pm 0.4$; and $K_i = 2.5 \pm 0.8 \times 10^{-6}$ M (n=6), $n_{app} = 3.8 \pm 0.7$. These differences were not related to culture conditions or age, and may reflect properties of multiple glycine receptor populations.
(Supported by NSF #BNS 8413780.)

419.12

SINGLE CHANNEL AND SYNAPTIC CURRENTS RECORDED IN NEURONS OF MAMMALIAN BRAIN AND SPINAL CORD SLICES.
A. Konnerth, T. Takahashi, F. Edwards and B. Sakmann (SPON: European Neuroscience Association). Max-Planck-Institut für biophys. Chemie, 3400 Göttingen, FRG.

The patch clamp technique (Hamill et al., *Pflügers Archiv 391, 1981*) has been applied to neurons in thin (100-130 μm) slices of rat brain and spinal cord. In this preparation neurons can be visually identified by using Nomarski water immersion optics. In order to apply the patch clamp method, single (synaptically connected) neurons were partially exposed by removing mechanically the covering cell debris. This "cleaning" procedure did not involve the use of proteolytic enzymes.

In spinal cord slices, electrical stimulation of interneurons evoked in motoneurons large (up to 2 nA) inhibitory postsynaptic currents (i.p.s.c.s) (V_h=-50 mV, $[Cl_i]=[Cl_o]$= =140 mM, 22-24 °C). The i.p.s.c.s were reversibly blocked by strychnine (2-5 μM) and reversed at the Cl^- reversal potential and were therefore identified as being mediated by glycine. Miniature i.p.s.c.s (mean amplitude ~40 pA) recorded in the presence of TTX had a steep onset (half rise time ~0.5 ms) and a slow decay which could be fitted by the sum of two exponentials (τ_1=4.6±1.2 ms, τ_2=25.8±10.0 ms). Glycine-mediated single channel currents, registered in excised outside-out patches in the same preparation, showed multiple conductance states. The two most frequently occurring states (2.3 and 2.7 pA at -50 mV) had life-time components (τ_1=2.5±1.4 ms, τ_2=27.5±8.3 ms and τ_1=2.5±0.9 ms, 26.0±11.5 ms, respectively) comparable to those obtained for the miniature i.p.s.c.s. These results indicate that the time course of the i.p.s.c.s reflects the gating properties of glycine receptor channels and that only 15-20 channels are activated by a single packet of inhibitory transmitter.
(Supported by DFG grant SFB236, A14).

DEGENERATIVE DISEASE: OTHER I

420.1

VASOACTIVE INTESTINAL PEPTIDE (VIP) AND CHOLECYSTOKININ (CCK) IN QUINOLINATE-LESIONED RATS AND HUNTINGTON'S DISEASE POSTMORTEM BRAIN
M.F. Mazurek, M.F. Beal, K.J. Swartz, S.F. Knowlton*, P. Patel* and J.B. Martin. McMaster Univ. Med. Ctr., Hamilton, ONT. and Mass. General Hosp., Boston, MA.

Intrastriatal injections of quinolinate (QUIN) can produce lesions which mimic many of the neurochemical and histopathological characteristics of Huntington's disease (HD). We studied levels of VIP and CCK by radioimmunoassay in chronic QUIN-lesioned Sprague-Dawley rats and in HD postmortem brain. Levels of VIP were elevated 3- to 4-fold in HD striatum, and 4- to 5-fold in QUIN-lesioned striatum. CCK was mostly normal in HD striatum as compared with a 2-fold increase in QUIN-lesioned rats. In cerebral cortex CCK immunoreactivity was elevated by 56-123% in all 13 areas that we examined, while VIP was increased to a lesser extent. There was no relationship between cortical peptide levels and the degree of striatal atrophy. QUIN-lesioned rats had normal cortical concentrations of CCK and VIP.

These results suggest that (1) a chronic QUIN lesion in the rat can produce changes in striatal VIP immunoreactivity that closely resemble those observed in HD; and (2) HD cerebral cortex contains increased levels of CCK immunoreactivity that are probably not adequately explained on the basis of abnormal feedback from the striatal-pallidal-thalamic circuit.

420.2

PATCH-MATRIX DISTRIBUTION OF CHOLECYSTOKININ AND CYTOCHROME OXIDASE ACTIVITY IN NORMAL AND HUNTINGTON'S DISEASE STRIATUM.
RJ Ferrante, NW Kowall and EP Richardson Jr. Massachusetts General Hospital and Harvard Medical School, Boston MA 02114.

The normal human striatum can be subdivided into two compartments based on the distribution of neurochemical substances and projections. In Huntington's disease (HD), acetylcholinesterase (AChE) histochemistry shows persistence of this patch-matrix pattern despite marked atrophy and neuronal loss. We examined the distribution of cholecystokinin (CCK) and cytochrome oxidase (CO) in 6 HD brains and 6 age-matched controls to further characterize patch-matrix relationships in HD striatum.

Clusters of CCK immunoreactive fiber terminals were heterogeneously distributed in both control and HD striatum. This patchy distribution has not been previously reported in human striatum. Comparison with adjacent AChE stained sections showed that CCK distribution corresponds to low AChE patches. The density of CCK patches was not altered in HD and the ratio of CCK positive patches to negatively stained matrix was increased.

In rodent striatum CO activity is patchy and enriched in spiny neuron dendrites. We found CO activity to be unevenly distributed in normal human striatum with regions of high CO activity corresponding to high AChE matrix. In HD striatum CO activity was reduced, especially in the dorsal striatum. Thus extrinsic CCK immunoreactive fiber terminals in striatal patches are preserved while striatal matrix enriched in CO is depleted. These findings confirm the relative preservation of striatal patches and striatal afferent systems and relative sensitivity of spiny neurons to destruction in HD.

420.3

A CANDIDATE CLONE FOR RAT 3-HYDROXANTHRANILIC ACID OXYGENASE. B. Navia*, M. MacDonald*, A. McClatchey*, J. Gusella, E. Okuno[1] and R. Schwarz[1](SPON: C. Kohler). [1]Neurogen. Lab., Mass. Gen. Hosp., Boston, MA 02114 and Maryland Psych. Res. Ctr, Baltimore, MD 21228.

Quinolinate (QUIN) is an endogenous brain metabolite which can cause selective excitotoxic neuronal death when introduced intracerebrally in rats. The synthetic enzyme for QUIN, 3-hydroxyanthranilic acid oxygenase (3-HAO) has been purified from rat liver and its presence has been demonstrated in the mammalian brain. In order to further elucidate the biological role of 3-HAO in the brain, we have isolated a candidate cDNA clone for rat 3-HAO. Using a polyclonal antiserum raised against purified rat 3-HAO (J. Neurochem.49,771, 1987), we screened a rat cDNA library constructed in the λgt11 expression vector. Two candidate 3-HAO cDNA clones, BN1 and BN2, were obtained. Each clone contained an approximately 1.5 kb insert which was subcloned into pUC19 for subsequent analyses. DNA sequencing revealed that the two were identical and contained only part of the putative coding region for 3-HAO. A search for DNA homology revealed the sequence to be unrelated to any previously reported genes. Efforts are under way to isolate a full-length cDNA to determine the complete structure of the 3-HAO enzyme, and to provide a critical reagent for examining the expression of this gene in the central nervous system. Supported by USPHS grants NS16102, NS20509 and NS16367.

420.4

MUSCLE WEAKNESS IN THE MDX MOUSE. C.G. Carlson, R.V. Makiejus*, J.W. Krieger*. Dept. of Biology, Purdue Univ. Calumet, Hammond, IN. 46323

The 'mdx' mouse represents an X-linked recessive myopathy (Bullfield et. al., PNAS, 81, 1189,1984) which lacks the same gene product as that lacking in Duchenne muscular dystrophy (Hoffman et. al., Cell, 51,919,1987). Using a non-invasive method for measuring 'whole body tension'(WBT) in which forward pulling tensions are averaged, we report that both male (WBT=8.6+ .6 gm tension/gm body wt.;5wks-21 mos) and female mdx mice (obtained from Roswell Park Memorial Cancer Institute and inbred;10.5+.4;5wks-9mos) are significantly weaker ($p<0.01$) than control mice (males-13.5 + .5;females-14.3+ .3;5 wks-8 mos.). In vivo (under Avertin anesthesia) twitch tension measurements in gastrocnemius showed a significant reduction in adult mdx mice (mdx-0.18 + .02 gm tension/mg wet wt.;C57-0.23+.01; $p<.05$) which was positively correlated ($r=.91$) to the WBT values. Intracellular recordings from adult mdx diaphragm fibers revealed Miniature Endplate Potential amplitude distributions characteristic of fully mature junctions which (unlike early postnatal C57 junctions) were normosensitive to elevations in extracellular potassium. These results indicate a myogenic impairment of tension generation in mdx muscle which is not associated with an inability to form fully functional mature nerve terminals. Supported by an SR Award (PUC) and an XL Summer Faculty Award (Purdue Res. Found.).

420.5

VERBAL FLUENCY IN PROGRESSIVE SUPRANUCLEAR PALSY (PSP). S. T. Smith* (SPON: W. A. Wilson). MGH Neurolinguistics Laboratory; Boston, MA 02108

Studies of cognitive processing in PSP commonly report severely impaired performance on tests of verbal fluency (production of words in a semantic or letter category in a specified time). Since these tests tap a variety of cognitive abilities (e.g., initiation, concept formation, abstract thinking, language ability and processing speed), breakdown in any of these abilities can cause impaired performance. In PSP, aspects of processing associated with frontal lobe dysfunction may be implicated.

In a case study of a 65 yr. old PSP patient with impaired fluency, an auditory recognition test designed to complement the fluency test was administered. Experimental words were tested for inclusion in their letter and semantic categories (e.g., pilot: P words; occupation). A subset of words for which the initial phoneme could be represented by more than one graphemic option was also examined in relation to a foil category (e.g., physician: F words). The same word list was repeated for each category to provide the opportunity to observe perseverations. Results indicated largely intact performance on the experimental task, and thus suggest that for this patient at least, semantic and orthographic knowledge necessary for the fluency test are preserved. Interpretations that fluency impairments in PSP are associated with frontal lobe dysfunction are thus supported by these data.

420.6

NEUROFILAMENT GENE EXPRESSION IN ANTERIOR HORN CELLS OF AMYOTROPHIC LATERAL SCLEROSIS (ALS). P.M. Tran*, A.W. Clark, I.M. Parhad. University of Calgary, Calgary, Alberta, Canada.

Studies in ALS have suggested an impaired production of RNA in surviving anterior horn cells (ahcs) and accumulation of neurofilaments (Nfs) in the proximal axons of ahcs. We evaluated the gene expression of the light neurofilament subunit (Nf-L) in 3 ALS and 3 control spinal cords. Neuronal counts showed approximately 50% loss of ahcs in the ALS cases. The area of individual ahcs was decreased in the ALS cases by approximately 25%. Total RNA recovery from the spinal cords was similar in ALS and control cases ($p>0.1$;). To date, RNase protection assays using an Nf-L riboprobe (JP Julien) have shown no difference in amount of Nf-L mRNA in ALS as compared to control cases. In situ hybridization, using the Nf-L probe revealed the expected direct correlation between neuronal size and Nf-L grain density in the control ahcs, but there was a loss of this correlation in ALS. Small ahcs in the ALS cases had an increased Nf-L grain density when compared with neurons of the same size in controls. These results suggest that Nf-L mRNA production is relatively well maintained in surviving ahcs in ALS.

420.7

THE EXPRESSION OF SPECIFIC PURKINJE CELL ANTIGENS IN TUMOR TISSUE FROM PATIENTS WITH PARANEOPLASTIC CEREBELLAR DEGENERATION. H.M. Furneaux*, D. Barbut*, F. Yee* and J.B. Posner. Dept. of Neurology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

Paraneoplastic cerebellar degeneration (PCD) is a rare remote effect of cancer which is characterized clinically by subacute onset of ataxia, dysarthria, nystagmus and pathologically by a diffuse loss of Purkinje cells. The occurrence of autoantibodies which recognize two specific Purkinje cell antigens (62 kd and 34 kd) in the sera of PCD patients suggests an autoimmune etiology. The link between the generation of these autoantibodies and the occurrence of systemic cancer is not clear. We have now demonstrated (by Western blot analysis using human and rabbit antibodies) the expression of both the 62 and 34 kd PUrkinje cell antigens in tumor tissue from PCD patients. These findings therefore support the hypothesis that paraneoplastic cerebellar degeneration is caused by an immune response to cerebellar Purkinje cell antigens provoked by their inappropriate expression in tumor tissue.

420.8

A PRIMATE MODEL OF CEREBROVASCULAR DISEASE: BEHAVIORAL AND NEUROPATHOLOGICAL STUDIES. M.B. Moss, T. Kemper*, D.L. Rosene, S. Prusty* and W. Hollander*. Boston University School of Medicine, Boston, MA 02118.

The behavioral and neuropathological consequences of hypertension and atherosclerosis were assessed in male cynomolgous monkeys that received coarctation of the thoracic aorta, alone (Group Hyp, N=3) or in combination with maintenance on an atherogenic diet (Group Hyp + Ath, N=5) for 12 months prior to testing. Post-treatment performance on a visual recognition memory task was compared to that of unoperated animals maintained on an atherogenic diet (Group Ath, N=3) and unoperated control animals maintained on a normal diet (Group N, N=4). Performance by the Hyp + Ath and Hyp groups, not significantly from each other, was impaired relative to the N and Ath groups. Further, two animals in the Hyp + Ath showed disproportionate impairment. In contrast, performance by animals in Group Ath did not differ significantly from Group N on the recognition task. Initial histological assessment revealed multifocal neuropathologies in monkeys in the Hyp+Ath and Hyp groups, including perivascular hemorrhage, mineral deposits, demyelination and ischemic infarction. The data suggest that hypertension alone, and perhaps to a greater extent when combined with an atherogenic diet, can produce marked impairment in CNS function.
(Supported by Grant HL13262)

420.9

REGIONAL CEREBRAL BLOOD FLOW ABNORMALITIES IN EARLY AIDS DEMENTIA COMPLEX MEASURED WITH 123-I-IMP SPECT. J. M. Mountz*, N. M. Speed*, K. Adams*, J. A. Schwartz*, M. D. Gross* D. G. Ostrow*. (SPON:D. E. Kuhl). University of Michigan Medical Center, Ann Arbor, Michigan 48109.

A frequent complication of acquired immune deficiency syndrome (AIDS) is dementia. We evaluated 7 AIDS patients (age 28-55, all males) for evidence of AID dementia complex (ADC). The assessment included a psychiatric evaluation, neuropsychological(NP) testing, Computed tomography (CT), and 123-I-iodoamphetamine single photon emission computed tomography (SPECT). Six of the 7 patients exhibited cognitive (5 with memory loss) or behavioral (2 with new onset of hypomania) abnormalities on psychiatric examination. NP testing showed general deficits, but none of the cases exhibited sufficient impairment to be classified as dementia. The SPECT scans showed marked abnormality in two cases: One case demonstrating diminution of tracer uptake in the posterior temporal parietal region (posterior/anterior =.81), and the other showed marked R/L asymmetry in the subcortical region (asymmetry= 1.17). In three additional patients there was asymmetric tracer uptake in the subcortical brain region on the SPECT scan (average=1 .13). CT scans were normal in all 7 cases.In conclusion, SPECT finding showed subcortical abnormalities in 4 out of 7 patients. This finding implies functional brain imaging with SPECT may be a useful method to follow ADC progression and to evaluate therapeutic agents.

EXCITATORY AMINO ACIDS IX

421.1

POLYAMINE DEPENDENCE OF NMDA RECEPTOR-MEDIATED Ca^{2+} FLUXES AND TRANSMITTER RELEASE FROM RAT HIPPOCAMPUS. F. Siddiqui*, Z. Iqbal and H. Koenig*. Neurology Dept., Northwestern University Medical School and Neurology Service, VA Lakeside Medical Center, Chicago, IL 60611.

The N-methyl-D-aspartate (NMDA) receptor is well characterized with respect to studies on excitatory effects of amino acids. In rat hippocampal synaptosomes,within 15 sec, 50-100 µM NMDA caused >3.5-fold increase in $^{45}Ca^{2+}$ influx, >3-fold increase in Ca^{2+} efflux and a 5-fold increase in the release of norepinephrine (NE) from synaptosomes. The NMDA-stimulated Ca^{2+} fluxes and NE release were inhibited by the specific NMDA-receptor antagonist, D-2-amino-5-phosphopentanoate, showing receptor mediation. The activity of the polyamine (PA) synthesis regulating enzyme, ornithine decarboxylase (ODC), increased 2- to 3-fold within 15-30 sec of NMDA exposure with a concomitant >1.5-fold elevation in putrescine concentration. The specific ODC inhibitor, α-difluoromethylornithine (DFMO), blocked NMDA-stimulated Ca^{2+} fluxes and NE release. DFMO inhibition was negated by the incorporation of putrescine in the incubation medium. Thus NMDA-controlled stimulation of synaptic transport functions appear to be dependent on PA which serve as messengers mediating cellular responses by enhancing Ca^{2+} fluxes needed for PA and Ca^{2+} dependent processes. (Supported by the VA and NIH grants HL 26835 and NS 18047)

421.2

EXCITATORY AMINO ACID EVOKED RELEASE OF -AMINOBUTYRIC ACID FROM CULTURED RETINAL NEURONS. H.-D. HOFMANN*, (SPON: G.B. Koelle), Max-Planck-Institut for Brain Research, 6000 Frankfurt, Fed. Rep. Germany

In dissociated neuronal cultures prepared from 8-day-old chick embryo retina, approximately 40% of the neurons accumulated ^{3}H-GABA via a high affinity uptake system. In contrast to depolarization by elevated KCl concentrations (56mM), glutamate, N-methyl-D-aspartate (NMDA), kainate and quisqualate were highly effective in eliciting the release of preloaded GABA, with half maximum effects at concentrations of 8µM, 80µM, 20µM and 2µM, respectively. Quisqualate evoked release was half of that observed in the presence of the other agonists. Autoradiographic analysis indicated that half of the ^{3}H-GABA accumulating cells did not respond to quisqualate, while glutamate, NMDA and kainate stimulated more than 95% of these cells. In agreement with electrophysiologically established characteristics of excitatory amino acid receptors, the NMDA receptor antagonists 2-amino-5-phosphonovalerat and MK 801, and high concentrations of Mg^2 (2-5mM) specifically inhibited the effects of NMDA without affecting responses to kainate and quisqualate. ^{3}H-GABA release, evoked by any of the agonists, was independent of external Ca^2 concentrations, but was abolished in the absence of Na . The results suggest that both NMDA and non-NMDA receptors that can trigger the release of preloaded GABA by a Ca^2 independent mechanism are present on the GABA accumulating retinal neurons.

421.3

GABA RELEASE EVOKED BY EXCITATORY AMINO ACID RECEPTOR ACTIVATION IN TYPE-2 ASTROCYTES IS INDEPENDENT OF cGMP FORMATION. G. Levi* and V. Gallo. (SPON: S. Denis-Donini) Neurobiology Section, Pathophysiology Laboratory, Istituto Superiore di Sanità, 00196 Roma, Italy.

Kainate (KA) (5-100 µM), quisqualate (QA) (5-50 µM) and glutamate (20-100 µM) stimulated ^{3}H-GABA release from type-2 astrocytes (AS) present in serum-containing cultures obtained from 8-day postnatal rat cerebella. Kynurenate (20-200 µM) largely antagonized the effects of KA and glutamate, but not that of QA. The releasing effects observed were totally Na^+-dependent, and partially Ca^{2+}-dependent in the case of KA. The releasing effect produced by 50 µM KA was largely prevented by an equimolar concentration of QA. However, 2 µM QA (ineffective by itself) generally potentiated the releasing action of KA. Cerebellar bipotential glial progenitor cells (precursors of type-2 AS) responded to KA and QA similarly to type-2 AS, and so did cultures enriched in type-2 AS obtained from neonatal cerebral cortex. The releasing effect of excitatory amino acid agonists did not appear to be related to a stimulation of cGMP formation. In fact, the level of cGMP was not significantly increased when cerebellar AS cultures were exposed to KA or QA. The lack of effect of these compounds was not due to the absence of guanylate cyclase. When the enzyme was activated by nitroprusside (200 µM) the level of cGMP increased up to 100 folds in cultures containing type-2 AS and much less in cultures devoid of type-2 AS, but ^{3}H-GABA release was unaffected. Immunofluorescence experiments using anti-guanylate cyclase antibodies showed a substantially stronger positivity in type-2 AS, glial precursors and oligodendrocytes than in type-1 AS. The excitatory amino acid agonists tested did not appear to interfere with the membrane carrier mediating GABA transport. It is concluded that type-2 AS and their precursors express two subtypes of excitatory amino acid receptors coupled to neurotransmitter release, but not to the synthesis of cGMP.

421.4

REGULATION OF EXCITATORY AMINO ACID(EAA)-INDUCED PHOSPHOINOSITIDES TURNOVER IN PRIMARY CULTURE OF RAT CEREBELLAR GRANULE CELLS BY γ-AMINOBUTYRIC ACID (GABA). Onnfoh Yu* and De-Maw Chuang (Sponsor: Ted H. Chiu). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Wash., D.C. 20032.

Primary culture of 8-day-old rat cerebellar neurons consists 95% of granule cells (GC) and have EAA receptors of the the kainate (Ka), N-methyl-D-aspartate (NMDA), and quisqualate (Quis) subtypes. Activation of these receptors stimulates the hydrolysis of phosphoinositides (PI) in a Mg^{++}-sensitive (NMDA) and non-Mg^{++}-sensitive (Ka, Quis) manner. This study investigated the effect of 7-day exposure of GC to 50 µM GABA on PI hydrolysis induced by L-glutamate (L-Glu), Ka, NMDA, and Quis in Mg^{++}-free physiological saline solution with 20 mM Li^+. GABA pretreatment produced a significant increase in the efficacies of L-Glu, NMDA, and Quis to stimulate [^{3}H]-inositol monophosphate accumulation in GC prelabeled with [^{3}H]-myo-inositol. There was no increase in their EC_{50}. The PI hydrolysis induced by Ka was not affected by this GABA pretreatment. This suggested that 7-day GABA pretreatment produced a selective up-regulation of the PI response mediated by NMDA and quisqualate receptor subtypes. (This work was done while O.Y. was stationed at NIMH as a Nat. Res. Council resident Res. Assoc).

421.5

STIMULATION OF INOSITOL PHOSPHOLIPID HYDROLYSIS BY GLUTAMATE IN GLIAL CELLS: A POSSIBLE ROLE IN THE REGULATION OF GROWTH AND DIFFERENTIATION

F. Nicoletti*, D.F. Condorelli*, G. Magrì*, F. Ingrao*, V. Bruno*, M.V. Catania* and R. Avola* (SPON: F. Drago) Institutes of Pharmacology and Biochemistry, Catania Univ. Sch. of Med., Catania, Italy.

Excitatory amino acid receptor agonists added to glial cells in primary culture increased inositol phospholipid (PI) hydrolysis and reduced [^{3}H]thymidine incorporation with the following rank order of potency: quisqualate > glutamate > ibotenate > kainate >> NMDA. Twelve hour-pretreatment of glial cells with epidermal growth factor (EGF) (10 ng/ml) increased the potency of glutamate and quisqualate in enhancing PI hydrolysis and reducing [^{3}H]thymidine incorporation, respectively. After pretreatment with EGF, PI hydrolysis was maximally stimulated by concentrations of glutamate (5 uM) that were otherwise devoid of activity. In addition, it appears that activation of glutamate receptors induces changes in glutamine synthetase activity, a biochemical marker of glial differentiation. We hypothesize that activation of specific metabolotropic glutamate receptors activates inositol phospholipid hydrolysis and reduces proliferation of glial cells in primary cultures.

421.6

KYNURENIC ACID CONTENT IN THE RAT BRAIN INCREASES DURING THE DEVELOPMENT AND THE AGING PROCESSES AND AFTER THE ADMINISTRATION OF PRECURSORS.

F. Moroni, P. Russi,* V. Carla'*. Dept. of Pharmacology, Univ. of Florence, Viale Morgagni 65, 50134, Florence, Italy.

The brain content of kynurenic acid (KYNA), a tryptophan metabolite which acts as an antagonist of the excitatory amino acid receptors, was measured using an original method based on ion exchange chromatography and HPLC. (Carla' et al. Analyt. Biochem.169: 89-94, 1988). In newborn animals brain KYNA content was extremely low (15±3 pmol/g protein, mean±SE), but an increase of twenty times occurred during the first 60 days of life. After sexual maturation brain KYNA content continued to increase, reaching values of 747±116 pmol/g protein in 18 month old animals. Aging did not change KYNA content in the liver or the kydney. The brain KYNA content also significantly increased after the administration of tryptophan (50-500 mg/kg i.p.) or of its keto analogue: indolpyruvic acid (IPA 50-500mg/Kg i.p.). Indirect evidence suggests that IPA may be directly metabolised to KYNA and that IPA administration may be considered as a new approach to antagonize "in vivo" excitatory amino acid receptors. Several pharmacological actions of IPA: sedation, analgesia and anticonvulsive effects, may be explained by its metabolism to KYNA.

421.7

DIFFERENCES IN AGONIST AND ANTAGONIST RECOGNITION SITES OF THE NMDA-SENSITIVE GLUTAMATE RECEPTOR.

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Several lines of evidence point at 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) as a competitive antagonist of the N-methyl-D-aspartate (NMDA)-sensitive glutamate recognition site. These include the inhibition of [^{3}H]glutamate binding, a similar distribution of [^{3}H]CPP and [^{3}H]glutamate binding in rat brain, and anticonvulsive actions of CPP. In our studies with rat brain synaptic membranes the specific binding of both [^{3}H]CPP and [^{3}H]glutamate was totally inhibited by 1 mM CPP, glutamate or 2-amino-5-phosphonovalerate (APV). However, interactions with modulatory sites present in the NMDA-sensitive receptor domain discriminate between CPP and glutamate recognition sites. Phencyclidine (PCP), a negative allosteric modulator, which fails to affect [^{3}H]glutamate binding, increases the affinity of CPP for its recognition site. D-serine, which acts at a positive modulatory site for the binding of [^{3}H]glutamate, downregulates [^{3}H]CPP binding. In both cases D-serine affects the affinity for the two ligands without changing the number of recognition sites. These data indicate that glutamate and CPP may bind to two different transitional states of the glutamate recognition site, inducing different conformations of the receptor complex.

421.8

FURTHER STUDIES WITH DEXTROMETHORPHAN AS AN N-METHYL-D-ASPARTATE (NMDA) ANTAGONIST.

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Morphinans are part of a large series of compounds active at phencyclidine (PCP) receptors that are also NMDA antagonists (Church et al., Eur. J. Pharm., 111:185, 1985). Interest has been expressed in their therapeutic potential and in their mode of action. We have investigated the systemic potency and compared their in vitro actions with that of other PCP-like drugs.

An 80% reduction of NMDA excitation of rat spinal or brainstem neurones followed i.v. injection of dextrorphan (4-16mg/kg), PCP (0.2-0.5mg/kg) or ketamine (2-10mg/kg), whereas dextromethorphan (50mg/kg) only reduced NMDA excitation by about 20%. Quisqualate and kainate actions were not reduced by these drugs. Effects of dextrorphan and PCP were much longer lasting than that of ketamine.

On cortical wedges in vitro, IC50 values for reduction of NMDA were:- PCP 0.4uM, dextrorphan 3.2uM, ketamine 11uM and dextromethorphan 35uM. Compounds such as caramiphen, noscapine and phenytoin, ligands for a dextromethorphan/caramiphen binding site (Musacchio et al., Neuropharm., 26:997, 1987) were inactive as NMDA antagonists. With all four PCP-like drugs, development of the block of NMDA was agonist-dependent, a finding consistent with the view that they act at a similar site within the channel coupled to the NMDA receptor.

421.9

NMDA-COUPLED AND UNCOUPLED PCP RECEPTORS: TENTATIVE NEUROCHEMICAL EVIDENCE FOR PCP RECEPTOR SUBTYPES.

T.S. RAO* H.S. KIM, J. LEHMANN, L.L. MARTIN, P.L. WOOD. Res. Dept., Ciba Geigy Pharmaceuticals, Summit, NJ 07901 and G.D.SEARLE & CO.,CNS Dis. Res., c/o MONSANTO CO., St.Louis, MO 63198.

In vitro studies on striatal ACh release and ligand binding studies have indicated a high degree of correlation between N-methyl-D-aspartate (NMDA) and phencyclidine (PCP) receptors. In vivo electrophysiological studies and cGMP measurements also suggested a tight coupling between these two receptors, although receptor autoradiographic studies indicated that not all PCP receptors may be coupled to NMDA receptors. In the present investigation we have examined the effects of PCP-receptor agonists, (PCP, ketamine and MK-801) and of NMDA and sigma receptor agonists and antagonists (NMDA and CGS 19755, ditolylguanidine (DTG) and rimcazole respectively) on mesocortical dopamine (DA) metabolism. PCP-receptor agonists markedly increased DA metabolism and release only in mesocortical regions without changing the striatal DA metabolism. The changes in the mesocortical DA metabolism exhibited stereospecificity, dose and time-dependency. However, the agonists and antagonists of NMDA and sigma receptors were ineffective in altering mesocortical DA metabolism. These data suggest that the PCP receptors which modulate mesocortical DA release, are not coupled to NMDA or sigma receptors.

421.10

MULTISTATE MODEL OF N-METHYL-D-ASPARTATE RECEPTOR FUNCTIONING.

D.C. Javitt and S.R. Zukin Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Phencyclidine (PCP) receptors have been shown to represent a site on the active conformation of the N-methyl-D-aspartate (NMDA) receptor complex. The interaction between PCP and NMDA receptors was studied using the selective PCP receptor ligand [^{3}H](+)-5-methyl-10,11-dihydro-5H-dibenzo[*a,d*]cycloheptene-5,10-imine maleate ([^{3}H]MK-801). Specific binding of [^{3}H]MK-801 was stimulated by excitatory amino acids (EAAs) with rank order of potency L-glutamate > NMDA > quisqualate > kainate confirming that this effect is mediated via NMDA receptors. The effect of EAAs on specific [^{3}H]MK-801 binding was blocked by the selective NMDA receptor antagonist D(-)2-amino-5-phosphonovaleric acid (D(-)AP5). D-serine and glycine increased the maximal efficacy but not the potency with which L-glutamate stimulated [^{3}H]MK-801 binding. The effect of D-serine and glycine was not inhibited by strychnine, suggesting that this potentiation is mediated via a non-strychnine-sensitive glycine receptor associated with the NMDA receptor complex. Scatchard analysis revealed two discrete components of [^{3}H]MK-801 binding. Incubation in the presence of L-glutamate or D-serine significantly altered the apparent densities but not the affinities of the high and low affinity components of binding. Under baseline conditions, more than 95% of binding sites were of low affinity. Incubation in the presence of D(-)AP5 led to a decrease in the apparent densities of this low affinity component of binding. Incubation in the presence of L-glutamate led to an dose-dependent increase in the apparent density of both high and low affinity [^{3}H]MK-801 binding vs. baseline and an apparent increase in the total number of [^{3}H]MK-801 binding sites. Incubation in the presence of combined D-serine and L-glutamate led to an increase in the apparent density of high affinity [^{3}H]MK-801 binding compared with incubation in the presence of either L-glutamate or D-serine alone but did not lead to a further increase in the apparent total number of binding sites. These data suggest a multistate model of NMDA receptor functioning in which glycine-like agents potentiate the ability of EAAs to convert receptors from closed to open conformations.

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421.11

MAPPING OF A FROG BRAIN KAINIC ACID RECEPTOR WITH POLYCLONAL ANTIBODIES. Wenthold, R.J. Dechesne*, C.J., Hampson D.R., Wheaton*, K.D. and Oberdorfer*, M.D. Laboratory of Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD. 20892

We have recently purified a kainic acid receptor from frog (Rana pipiens) brain using ion exchange chromatography and domoic acid affinity chromatography (Hampson and Wenthold, J. Biol. Chem. 263:2500, 1988). The purified receptor has high and low affinity components with dissociation constants and inhibition constants similar to those of the membrane-bound or crude soluble receptor. Polyclonal antibodies were made in rabbits with injections of 15-30 micrograms of the affinity purified preparation. Western analyses of antibody binding to whole frog brain showed several closely-spaced reactive bands which migrated with the purified receptor (M_r = 48,000). Analysis of frog liver, heart and muscle showed no corresponding immunoreactive bands. An antibody binding assay showed that the frog brain kainic acid receptor was strongly recognized by the antibody. However, the antibody did not recognize kainic acid receptors from several other species.

For immunocytochemical analysis frog brains were fixed by perfusion or immersion with 4% paraformaldehyde. Immunoreactivity was found throughout the brain with heaviest staining in the telencephalon and lowest in brain stem. The immunocytochemical distribution closely followed the distribution of the receptor determined autoradiographically using ^{3}H-kainic acid bound to cryostat sections. At higher magnifications, kainic acid immunoreactivity showed a punctate labeling, often appearing to follow neuronal fibers. Cell soma labeling was not present.

421.12

DIFFERENT TARGET SIZES OF THE NMDA RECEPTOR CHANNEL SITES AND THE SIGMA OPIATE SITE IN RAT BRAIN. E.H.F. Wong and M. Nielsen[1]. Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, CM20 2QR, U.K.; [1]Psychopharmacological Research Laboratory, Sct. Hans Mental Hospital, DK-4000 Roskilde, Denmark.

It has previously been proposed that phencyclidine (PCP) and sigma opiates confer their psychotomimetic effects by acting on a common PCP/sigma site by virtue of their ability to generalise in a discriminative stimulus assay (Shannon, H.E., JPET 216: 543, 1983) and the similarity in pharmacological specificity when these sites were labelled by [^{3}H]-PCP and the prototypic sigma ligand (+)-[^{3}H]-SKF 10,047 (Mendelsohn et al, JPET 233: 597, 1985). Recent studies with more selective ligands for these two sites have highlighted a clear difference in their pharmacological specificity and regional distribution. The similarity in behavioural profile of these compounds are presumably due to their lack of selectivity.

We have investigated the two sites by measuring their molecular size utilizing the selective and potent N-methyl-D-aspartate/PCP receptor channel ligand [^{3}H]-MK-801 (Wong et al, PNAS 83: 7104, 1986) and the selective sigma ligand [^{3}H]-di-tolylguanidine (DTG) (Weber et al, PNAS 83: 8784, 1986). High energy inactivation of frozen rat cortex was used to determine the molecular target sizes of thiencyclohexylpiperidine-sensitive [^{3}H]-MK-801 binding and haloperidol-sensitive [^{3}H]-DTG binding giving values of 65900 ± 3500 daltons and 16700 ± 3700 daltons respectively.

These results have provided further evidence for a difference in the molecular characteristic of these two receptor binding sites.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION I

422.1

SINGLE OR REPEATED MILD STRESS INCREASES SYNTHESIS AND RELEASE OF HYPOTHALAMIC CORTICOTROPIN-RELEASING FACTOR (CRF). D.A. Haas and S.R. George, Departments of Medicine and Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8, Canada.

Since one of the prime mediators of the stress response is CRF, we examined the effect of a specific mild stress on CRF in hypothalamus of adult male rats. CRF immunoreactivity (CRF-ir) was determined using a rat CRF-specific RIA, and the relative contributions of synthesis and release assessed. A single 5 minute restraint significantly increased CRF-ir in the median eminence 24 hrs later compared to controls ($p<0.025$), with no change detected earlier. Plasma ACTH, an index of CRF release, was significantly elevated within 15 minutes of restraint ($p<0.025$). Repetition of a mild stress daily for 9 days ($p<0.01$), or a single episode of handling ($p<0.05$), both resulted in significantly increased CRF-ir in hypothalamus 24 hrs later. This change could be due to either increased CRF synthesis or inhibition of release. In order to investigate the mechanism involved, the 5 min restraint was repeated while protein synthesis was blocked with the protein synthesis inhibitor anisomycin. This resulted in significantly decreased CRF-ir in the median eminence 24 hrs later compared to vehicle-injected stressed ($p<0.005$) or to anisomycin-injected unstressed rats ($p<0.025$). Therefore, inhibition of protein synthesis prevented the expected post-stress increase in CRF-ir. These data show that mild stress increased net hypothalamic CRF content as a result of the balance between augmented synthesis and augmented release.

422.2

CHRONIC CORTICOTROPIN-RELEASING FACTOR (CRF) ALTERS RAT BRAIN BIOGENIC AMINE RECEPTORS. L.J. Grimm & D.C. Perry. Department of Pharmacology, George Washington Medical Center, Washington, D.C. 20037

CRF is believed to act as a CNS neurotransmitter, being released by stressful stimuli and initiating a multicomponent response to stress in the CNS and periphery. Stress is hypothesized to play an important role in depression: depressed patients often have a disrupted ability to regulate their CRF-ACTH-cortisol system, and exhibit abnormally high CSF levels of CRF. To examine effects of chronically high levels of CRF, rats were given i.c.v. CRF (5 ug in 5 ul saline) or saline twice daily for 10 days. Binding to several types of biogenic amine receptors was measured in different regions, and norepinephrine-stimulated cAMP was measured in cortical slices. Large increases were seen in 5HT-1 (but not 5HT-1a) binding in frontal cortex (64%), hippocampus (45%) and hypothalamus (19%) and in 5HT-2 binding in cortex (51%), striatum (40%) and cerebellum (50%). Alpha-1 adrenergic binding was unchanged except for an 18% increase in cortex. Beta adrenergic binding was only modestly affected, increasing in cortex (10%) and cerebellum (9%) and decreasing in pons/medulla (9%). Maximal level of norepinephrine-stimulated cAMP was increased. Thus chronic CRF leads to profound changes in binding and function of brain biogenic amine receptors, indicating a possible role for this peptide in depression. Supported by NIH 2SO7RR-05359-26.

422.3

STRESS-INDUCED FALL IN BLOOD PRESSURE IN HYPERTENSIVE RATS: STUDIES ON ENVIRONMENTAL, NEURAL AND NEUROENDOCRINE FACTORS. B.Bohus, J.M.Koolhaas, C.Nyakas and F.Hindriks (SPON: European Brain and Behaviour Society) Dept. of Anim.Physiology, Univ. Groningen, Haren, The Netherlands.

Rats proned for hypertension show a slight decrease in resting blood pressure (BP) a day after the stress of single social defeat (Fokkema, D.F. and Koolhaas, J.M., Physiol. Behav., 34: 33, 1985). The nature and mechanism of this unexpected stress response has been investigated in male rats with experimental (DOCA/salt) and genetic (spontaneous, SH) hypertension.

Stress of social defeat on 3 consecutive days led to a substantial fall of resting BP of DOCA/salt and SH rats, and a slight increase in WKy rats. This fall outlasted the stress for a few days. Stress of forced swimming, shuttle-box avoidance and escape training, unavoidable footshock or conditioned fear after a single unavoidable footshock led to a similar pattern of BP decrease in DOCA-salt hypertensives. Electrolytic destruction of the central nucleus of the amygdala prevented, while administration of the opioid antagonist before each expose to stress enhanced the fall of BP. It is concluded that the inverse cardiovascular response of hypertensive rats is (a) independent of the quality of the stressor stimulus in the dimension of controllability and predictability; (b) it involves limbic-forebrain neural mechanism, and (c) opioid system(s) play(s) a protective role in its induction. (Supported in part by the Dutch Heart Foundation, grant no. 84.002.)

422.4

OPIATES, CRH, AND OTHER NEUROMODULATORS AS MEDIATORS OF FEAR-INDUCED BEHAVIORAL RESPONSES IN THE INFANT PRIMATE. N.H. Kalin and S.E. Shelton*. Dept. of Psychiatry, VA Hospital and Univ. Wisconsin-Madison, Madison, WI 53792.

In work with separated infant rhesus monkeys tested in the presence of humans, we established that morphine attenuates and naloxone increases distress vocalizations (DVs) without affecting activity levels. Diazepam increases activity, while CRH increases behavioral inhibition without sedating the separated infants. Subsequent work showed that, depending on the environment in which a monkey is separated, fear elicits calls for help, withdrawal, or aggressive behaviors. To study neurochemical mechanisms underlying fear-induced behaviors, we established behavioral paradigms to selectively enhance withdrawal or aggressive behaviors. Infants briefly separated from their mothers were placed alone (A), in the presence of a human who did not maintain eye contact (NEC), or in the presence of a human who maintained continuous eye contact (EC). A infants showed high levels of locomotion and DVs. In comparison, EC increased and NEC decreased DVs ($p<.002$). Barking, an aggressive gesture occurring rarely in A or NEC, was evoked at high levels by EC ($p<.001$). Freezing and slowed locomotion, rare in A and EC, increased in NEC ($p<.001$). We are now assessing the role of endogenous opiate, CRH, and benzodiazepine systems in mediating fear-induced aggression elicited by EC and freezing behavior elicited by NEC.

422.5

EFFECTS OF ECT ON THE HPA AXIS: BASIC AND CLINICAL STUDIES. E.A. Young, M.K.-H. Schafer,* J. Herman, R. Day, S.J. Watson, and H. Akil. Mental Health Research Institute, University of Michigan, 205 Washtenaw Place, Ann Arbor, Michigan, 48109-0720.

Depression is accompanied by disturbance of the HPA axis. These abnormalities have been shown to normalize with effective treatment including ECT. However, ECT may have effects of its own on the HPA axis, since repeated seizures may act as a chronic stressor. Initial studies in patients compared the release of β-endorphin (βE) from the anterior pituitary with the first and last treatment. Overall, 11/17 individuals released more βE with the last treatment than with the first treatment. Those individuals who released less showed much shorter seizures with the final treatment than the first treatment. To explore the mechanism involved in HPA changes with repeated ECT, studies were undertaken in rats. In the rat, there is also a greater release with treatment #8 than with treatment #1. Measurement of the anterior pituitary content shows a 40% increase in βE in the chronic ECT rats. There is also an increase in CRF mRNA in the paraventricular nucleus of the hypothalamus. The adrenal weight is increased by 25%, and this results in a 3-fold increase in basal a.m. cortisol levels compared to unhandled control rats. There is also a shift in forms of βE-IR with repeated ECT. In rats, the 1st treatment releases βE over β-LPH in a ratio of 2:1. This is also the case in man. However, the eighth session releases predominantly β-LPH, with a ratio of β-LPH:βE of 2:1. In man, this shift in ratios does not appear to occur.

422.6

HUMAN CATECHOLAMINE RESPONSES TO STRESS AFTER DEXAMETHASONE, SCOPOLAMINE PLUS AMPHETAMINE, AND PLACEBO. R. L. Kohl. Universities Space Research Association, Space Biomedical Research Institute, Johnson Space Center, National Aeronautics and Space Administration, Houston, TX 77058.

Stress level was incrementally raised using a Staircase Profile Test (SPT) on a rotating chair assembly (Kohl, R. L., Aviat. Space Environ. Med., 58:125, 1987). Subjects made head movements out of the plane of rotation until stimulation of the vestibular system induced advanced nausea and multiple signs of autonomic system dysfunction (i.e., motion sickness). Dexamethasone (DEX) was loaded (3 mg/da) for 3 da and throughout a subsequent 5 da period of once daily SPTs. Scopolamine plus d-amphetamine (S/D, 0.4/5 mg) was administered 1.5 hr prior to 5 daily SPTs. Six blood samples were obtained prior to orally administered drug, and after drug on Monday and Friday, immediately before and after the SPT. Epinephrine (EPI) and norepinephrine (NE) levels generally rose following a SPT. DEX diminished or reversed the stress-induced rises in EPI and NE, particularly on Monday ($p<.05$). Poststress levels ranged from 30 to 65% of control. Lower pretest levels of NE in subjects receiving DEX indicated declining sympathoadrenal function ($p<.05$). S/D did not modulate catecholamine levels or responses to stressful motion. Because both drugs are effective antimotion sickness drugs, it follows that reduction of peripheral catecholamine levels or responses to stress probably does not underlie the therapeutic action of these agents.

422.7

SYMPATHETIC-ADRENAL MEDULLARY RESPONSE TO ACUTE FOOTSHOCK STRESS IN SHRs IS ASSOCIATED WITH HYPERACTIVITY TRAIT, NOT HYPERTENSION. E.D.Hendley, M.A.Cierpial* and R. McCarty*. Univ. Vermont, Burlington, VT 05405 and Univ. Virginia, Charlottesville, VA 22903.

Four genetically related, inbred Wistar-Kyoto rat strains were used to examine the sympathetic-adrenal medullary response to acute footshock stress. It had been shown previously (McCarty & Kopin, Physiol & Behav 21:567, 1978) that SHRs increase plasma catecholamines to higher levels than in WKY controls, in response to acute footshock. In this study we examined whether this sympathetic-adrenal medullary hyperreactivity is attributable to the hypertensive trait of SHRs or to the hyperactive trait that is also characteristic of SHRs. Two new inbred strains produced from a SHR X WKY cross are WK-HAs, that are hyperactive but not hypertensive, and WK-HTs, that are hypertensive but not hyperactive. These two strains plus SHR and WKY were subjected to acute footshock. Plasma catecholamines increased significantly higher in WK-HAs and SHRs than in WK-HTs and WKYs. Thus, sympathetic-adrenal medullary hyperreactivity is associated with the hyperactivity trait, common to SHR and WK-HA strains, and not to the hypertensive trait.
Supported by HL 29906, MH 00529 (R.M.) and Univ. Vermont College Medicine Research Committee (EDH).

422.8

RELEASE OF PROLACTIN AND CORTISOL FOLLOWING COCAINE CESSATION IN MEN. E.M. Dax, W.W. Weddington*, N.S. Pilotte and J.J. Jaffe* (SPON: J.C. Fernando), Addiction Res. Ctr., Nat'l Inst. on Drug Abuse, Baltimore, MD 21224.

The use of cocaine is thought to alter the dynamics of central dopamine secretion which in turn may affect the secretion of prolactin (PRL), a hormone that is released rhythmically throughout the day. Cortisol is also released rhythmically but is not thought to be under dopaminergic control. To assess if these rhythms were altered in the same manner by cocaine, plasma PRL and cortisol levels were evaluated q 2 hr over at least two 24-hr periods following acute cessation of cocaine usage in 7 men who were known cocaine users and 3 non-abuser controls on the same research ward. The mean 24-hr PRL was significantly higher ($p < 0.05$) in abusers (14.6 ng/ml) than in controls (9.8 ng/ml) from days 2-10 after cessation. These men were mildly hyperprolactinemic. No diurnal variation in PRL levels was observed for up to 10 days. Interestingly, there was no difference between the lowest circulating levels of PRL in these two groups, but the cocaine abusers attained significantly greater ($p < 0.01$) peak levels of PRL throughout the day than did controls. Over the study period, normal cortisol levels and diurnal rhythms were present ($p < 0.001$) and invariant from day to day. Thus, PRL secretion is affected during cocaine withdrawal but whereas cortisol is not.

422.9

IV VENTRICULAR MEDIATION OF THE ACTH RESPONSE TO CENTRAL NICOTINIC STIMULATION. S.G. Matta*, K.M. McAllen*, and B.M. Sharp* (SPON: F.J. Wilson), Mpls. Med. Res. Fdn. and Dept. Med., Hennepin Cty. Med. Ctr. and Univ. Minnesota, Mpls, MN 55415.

Injection of a low dose of nicotine (N; 1 ug) into the III ventricle of the rat brain or of a relatively high dose (10ug) into the PVN rapidly elevates plasma ACTH to levels produced by peripherally administered N. To localize the site of action of N, current studies targeted the IV ventricle. Rats with chronic intracerebroventricular (icv) cannulae and chronic jugular cannulae, were given 0.5 ul N or buffer (B) icv; ACTH values (pg/ml) are mean ± sem:

	0 min	3 min	7 min	15 min
Buffer	26±5	24±6	27±10	40±13
N 0.25 ug	19±3	439±68	425±84	201±39
N 0.5 ug	17±4	680±96	902±168	403±85
N 2.5 ug	29±8	585±131	1064±126	615±69
N 5.0 ug	20±8	717±123	921±104	458±57

The N receptor antagonist mecamylamine (MEC, 1 mg/kg BW iv) significantly suppressed the ACTH response to N 0.25 ug icv by 70%; hexamethonium was ineffective. Moreover, MEC (40 ug) administered icv significantly suppressed the ACTH response to peripheral N (0.03 mg/kg iv). Therefore, sites mediating ACTH release following either central or peripheral administration of N may be found adjacent to the IV ventricle. (Supported by DA 03977).

422.10

A PERIPHERAL CORTICOTROPH STIMULATING FACTOR (PCSF) IS RELEASED FROM INFLAMED TISSUE IN RATS. K. M. Hargreaves* and A. H. Costello* (Spon: J. Hylden), NAB, NIDR, NIH, Bethesda, MD 20892

We have previously reported that circulating levels of immunoreactive beta endorphin (iB-END) are elevated during carrageenan (carra)-induced inflammation in rats (Abs. Soc. Neurosci. 12:374,1986). The present studies determined whether secretion of pituitary iB-END was due either to activation of central neuronal processes or to release of a PCSF from inflamed tissue. Inflammation was induced by sc injection of carra (3mg) into the plantar surface of hindpaws. Ninety min later subcutaneous perfusates of carra- and sal-treated hindpaws were collected (1ml/10min) in anesthetized rats using a coaxial system. Primary cultures of rat anterior lobe pituitary cultures were grown for 1 week prior to screening perfusates for releasing activity. Levels of iB-END were measured by RIA with data analyzed by ANOVA followed by Duncan's test. In an initial experiment, lesioning the sciatic and saphenous nerves one week prior to the study did not block the increase in circulating iB-END due to carra inflammation as compared to a sham surgery group (59.1±7.8 fm/ml and 40±6.0 fm/ml): both were significantly elevated over control levels (28.7±2.9 fm/ml; $p<.01$ for both). Pituitary cell culture secretion of iB-END due to the administration of carra perfusates (1318±188 fm/well) was significantly greater than secretion due to sal perfusates (328±32; $p<.01$) or to basal release (216±22 fm/well;$p<.01$). In a separate experiment, iB-END release due to carra perfusate (269±25 fm/well) was significantly greater than basal release (136±2 fm/well; $p<.01$) and was blocked in calcium-free media (130±2.6 fm/well;$p<.01$). The releasing activity was not due to a direct effect of carra or to substance P, CGRP, bradykinin or epinephrine. The results indicate that a PCSF in inflamed tissue stimulates iB-END release in the denervated rat and in cell cultures and suggests that hypothalamic and extrahypothalamic factors modulate secretion of iB-END in response to inflammation.

422.11

IDENTIFICATION OF INTERLEUKIN-1 RECEPTORS IN MOUSE PITUITARY CELL MEMBRANES AND AtT-20 PITUITARY TUMOR CELLS. Daniel E. Tracey* and Errol B. De Souza (SPON: S. R. Franklin). Hypersensitivity Diseases Res., The Upjohn Company, Kalamazoo, MI 49007 and Neuroscience Branch, NIDA, Addiction Research Center, Baltimore, MD 21224.

The cytokine interleukin-1 (IL-1) has a variety of effects in the brain, including stimulation of the hypothalamic-pituitary-adrenal axis. Whether IL-1 induces adrenocorticotropic hormone secretion by direct stimulation of cells in the pituitary or indirectly via hypothalamic stimulation of corticotropin releasing factor is controversial. We examined this question by measuring the binding of ^{125}I-labeled recombinant human IL-1α (rhIL-1α) to cell membranes from whole mouse or rat pituitary glands and brain regions. Specific binding of labeled IL-1 to pituitary membranes from C57BL/6 and other strains of mice was ten-fold higher than binding to Sprague-Dawley rat pituitary cell membranes, much higher than to mouse brain regions, including hypothalamus, and comparable to binding in mouse spleen. Labeled IL-1 also bound specifically to whole cells or membranes from the AtT-20 mouse pituitary tumor cell line and the EL-4 6.1 mouse thymoma cell line. The binding of labeled IL-1 to mouse pituitary, AtT-20 and EL-4 membranes was temperature dependent, saturable, and of high affinity with a K_D of 40-60 pM and B_{max} values of 9 (pituitary), 8 (AtT-20) and 116 (EL-4) fmoles/mg protein (570 and 6300 sites/cell for AtT-20 and EL-4 cells, respectively). Labeled rhIL-1α binding was specifically inhibited by rhIL-1α, rhIL-1β and an analog, rhIL-1β+, in parallel with their relative bioactivities, but not by an inactive IL-1 peptide or by unrelated peptides. These studies provide the first demonstration of IL-1 receptors in mouse pituitary and AtT-20 cell membranes with properties indistinguishable from the well-characterized EL-4 IL-1 receptors.

422.12

ACTIVATION OF THE "PERIPHERAL" BENZODIAZEPINE RECEPTOR INDUCES SECRETION OF RAT HYPOTHALAMIC CORTICOTROPIN-RELEASING HORMONE (CRH) IN VITRO. A.E. Calogero*, W.T. Gallucci*§, R. Bernardini, S.J. Listwak*§, P.W. Gold*§ and G.P. Chrousos DEB, NICHD and BPB§, NIMH, Bethesda, MD 20892

"Peripheral" benzodiazepine (pBZD) receptors are present in the pituitary, adrenal gland and testes. Although the role of these receptors in endocrine glands is largely unknown, it appears that pBZD receptor agonists modulate endocrine responses. Ro5-4864, a pBZD receptor agonist, inhibits β-endorphin secretion from AtT-20 cells, a mouse pituitary tumor cell line, and that the same agent increases basal and hCG-stimulated testosterone secretion in vitro. In the present study, we examined the involvement of the pBZD receptor in the secretion of immunoreactive CRH (iCRH) by explanted rat hypothalami. After explantation and overnight preincubation in medium 199 (M199), single hypothalami were incubated in M199 for 60 min, followed by a 40 min stimulation with graded concentrations of Ro5-4864 (Hoffman-LaRoche, Nutley, NJ) or Ro5-4864 plus the pBZD receptor antagonist PK 11195 (Dr. Le Fur, Pharmuka, France). The viability of each explant employed was tested, by examining the iCRH response to 60 mM KCl-induced depolarization. Ro5-4864 stimulated iCRH secretion in a dose-dependent fashion ($p<0.001$, ANOVA followed by Duncan multiple range test) with an ED_{50} of 3.5×10^{-8} M. The stimulatory effect of 10^{-7} M Ro5-4864 was completely antagonized by equimolar concentrations of PK 11195 ($p<0.001$, Student t test).

We conclude that activation of the pBZD receptors induces secretion of hypothalamic CRH in vitro. We speculate that the pBZD receptors may play a role in the in vivo regulation of the hypothalamic-pituitary-adrenal axis.

RECEPTOR MODULATION AND REGULATION III

423.1

DENERVATION TRIGGERS TRANSCRIPTIONAL ACTIVATION OF SKELETAL MUSCLE ACETYLCHOLINE RECEPTOR GENES. Huey-Jen Tsay* and Jakob Schmidt (SPON: S. Yazulla). Dept. of Biochemistry, State University of New York at Stony Brook, Stony Brook, New York 11794.

Transcriptional activity of acetylcholine receptor subunit genes was investigated in innervated and denervated chick skeletal muscle. The sciatic nerve of 3-day old White Leghorn chicks was sectioned unilaterally; after various intervals, nuclei were isolated from operated and control (sham operated) animals, and run-on assays performed. Nuclei were incubated with ^{32}P-UTP, and total RNA extracted and hybridized onto filters containing an excess of subunit-specific DNA. Specific transcripts were detected by autoradiography and quantitated densitometrically. A sharp increase in transcriptional activity was observed which began about 12 h after the operation and peaked 1.5 days later when transcriptional rates reached approximately 8; 6; and 5fold control levels for the alpha, gamma, and delta subunit genes, respectively. A substantial decline, to less than 2fold control levels was seen by the fourth day after the operation.

These results indicate that a denervation signal reaches the genome to induce receptor expression.

423.2

REPEATED RESERPINE TREATMENT UP-REGULATES D-1 RECEPTOR - ASSOCIATED ADENYLATE CYCLASE WITHOUT CHANGING THE DENSITY OF 3H - SCH 23390 BINDING. C. Missale, E. Nisoli*, S. Sigala*, M. Memo, P.F. Spano. Inst. Pharmacol. Brescia Univ. Sch. of Med., Brescia, Italy.

The present study investigates D-1 receptor plasticity using the 5 day treatment with reserpine as an experimental model. Male SD rats were given reserpine (1mg/Kg; s.c.) for 5 days and killed on the 5th day 2h after the last injection. Striatal D-1 receptors were studied both with 3H-SCH 23390 and by measuring cAMP formation in response to the selective agonist SKF 82526. The responsiveness of adenylate cyclase (AC) to D-1 receptor stimulation was markedly increased after reserpinization (EC-50 = 5μM in controls and EC-50 = 1.8μM in reserpine-treated rats); no significant changes were found in 3H-SCH 23390 binding site density (Bmax = 319±28 fmol/mg prot in controls and Bmax = 312 ± 30 fmol/mg prot in reserpinized rats). In addition, formation of cAMP induced by GppNHp was markedly enhanced in DA-depleted rats, while the responsiveness of AC to forskolin or to increasing concentrations of Mg-ATP was unchanged.

423.3

NEUROTENSIN REDUCES THE AFFINITY OF DOPAMINE D-2 RECEPTORS IN MEMBRANES FROM THE NEOSTRIATUM AND SUBCORTICAL LIMBIC AREAS OF THE RAT. G. von Euler*, K. Fuxe, L. Agnati* and F. Benfenati*. Dept. of Histology and Neurobiology, Karolinska Inst., Box 60400, S-104 01 Stockholm, Sweden, and Dept. of Human Physiology, University of Modena, Italy.

The effects of neurotensin in vitro were analyzed on the binding characteristics of 3H-N-propylnorapomorphine (3H-NPA, a D-2 agonist in vitro) in membrane preparations of neostriatum and subcortical limbic areas (mainly nucleus accumbens and tuberculum olfactorium) in the rat. Under equilibrium conditions neurotensin increased the KD-value of 3H-NPA binding in a concentration related (0.3 - 100 nM) biphasic fashion with a peak increase at 3 nM to 122 ± 9 % in neostriatal membranes and to 129 ± 12 % in subcortical limbic membranes. The Bmax-value of 3H-NPA binding was not significantly affected by neurotensin in the areas analyzed. Kinetic analysis revealed that neurotensin reduced the binding of 3H-NPA within 5 minutes of the addition of the peptide. These results indicate that the neurotensin receptor interacts with the D-2 receptor within the plane of the membrane. The dose needed to induce maximal modulation of D-2 receptors is below the dose needed to saturate the neurotensin receptors. Thus, the likelihood for inducing homeostatic mechanisms, such as down-regulation of the neurotensin receptor is highly reduced. In conclusion, the ability of neurotensin to reduce the affinity of central D-2 receptors may be of importance for the ethiology and treatment of schizophrenia and tardive dyskinesias.

423.4

DIFFERENTIAL REGULATION OF ALPHA-1 ADRENERGIC RECEPTOR SUBTYPES BY ELECTROCONVULSIVE SHOCK AND RESERPINE. J.A. Blendy, L.J. Grimm, D.C. Perry, K.J.Kellar. Depts. of Pharmacology, Georgetown and George Washington Univ., Washington, DC 20007

Repeated administration of electroconvulsive shock as well as chronic treatment with reserpine increases alpha-1-adrenergic receptors as labeled by ^{3}H-Prazosin (Vetulani, et.al., Brain Res. 275:392, 1983, Stockmeier, et.al. Eur J Pharm, 139:259,1987). Recent studies indicate that ^{3}H-Prazosin labels both alpha-1a and alpha-1b subtypes with equal affinity, while ^{3}H-WB4101 labels primarily the alpha 1a subtype (Morrow and Creese, Mol. Pharm 29:321,1986). To determine whether these subtypes could be regulated differentially by either reserpine or ECS we treated rats for 10-12 days with ECS or for 15 days with reserpine (.5mg/kg, i.p.). Quantitative in vitro autoradiography confirmed homogenate binding studies in that ECS increased ^{3}H-Prazosin binding in most regions of the cortex by 18-46% and in the medial amygdala by 60%. ^{3}H-WB4101 binding, on the other hand, was unaffected by ECS in all regions of the brain analyzed except in the medial amygdala where it remained increased by a lesser (15%) but significant extent. Chronic reserpine treatment increased ^{3}H-Prazosin binding in the frontal cortex by 50% while ^{3}H-WB4101 binding was not changed by this treatment. Hence, in both homogenate and autoradiography studies, ECS appears to increase ^{3}H-Prazosin binding more than ^{3}H-WB4101 binding; while reserpine, in homogenate studies, increased ^{3}H-Prazosin binding but not ^{3}H-WB4101 binding. These studies suggest that the two subtypes may be differentially regulated.

423.5

SELECTIVE REGULATION OF β_2-ADRENERGIC RECEPTORS BY DEXAMETHASONE. K.A. Neve. VAMC, Portland, OR 97207.

Glucocorticoids modulate the response of some tissues to ß-adrenergic receptor agonists by increasing the density of ß-adrenergic receptors. It is not known to what extent tissue-specific effects of glucocorticoids may be accounted for by variations in (1) the proportions of β_1 and β_2 receptors and the sensitivity of each subtype to glucocorticoids, (2) the presence of glucocorticoid receptors, or (3) the presence of other glucocorticoid-modulated proteins. Treatment of L6 myoblasts with 1 nM dexamethasone (DEX) increased the density of β_2 receptors by 9%, from 507 to 554 fmol/mg of protein. The effect of DEX treatment was dose-dependent, with an increase of 30% observed after treatment with 1 µM DEX. The DEX-induced receptor proliferation was maximal within 12 hr of the onset of treatment. The C_6 cell line is a glioma-derived line that expresses both subtypes of ß receptors. Treatment of C_6 cells with 1 µM DEX for 16 hr selectively increased the density of β_2-adrenergic receptors from 25 to 37 fmol/mg of protein (50%). A smaller increase was observed after treatment with 10 nM DEX (31%). The DEX-induced proliferation of receptors was prevented by cycloheximide (100 µM). DEX had no effect on the density of β_1-adrenergic receptors. Thus, tissue-specific effects of DEX on ß-adrenergic receptors may be due to receptor subtype specificity.

423.6

PHOSPHORYLATION OF PURIFIED AND MEMBRANE µ-OPIOID RECEPTORS ATTENUATE FUNCTIONAL COUPLING TO Gi, BUT NOT GTPγS-SENSITIVE AGONIST BINDING

H. Ueda[1], H. Harada[1]*, Y. Wada[1]*, T. Katada[2]*, M. Ui[3]*, H. Takagi[1] and M. Satoh[1]. [1]Dept. Pharmacol., Fac. of Pharmac. Sci., Kyoto Univ., Kyoto 606, [2]Tokyo Inst. Tech., Yokohama 227, [3]Univ. of Tokyo, Tokyo 113, Japan.

The regulatory mechanism of opioid signal transduction by phosphorylation of purified and membrane bound µ-opioid receptor by cyclic AMP-dependent kinase (A-kinase) was studied. The A-kinase phosphorylation of synaptic membranes from rat brains attenuated the µ-agonist (DAGO)-stimulation of low Km GTPase, while did not change the GTPγS-sensitive high affinity agonist ([^{3}H]DAGO) binding. In reconstituted preparations of purified Gi with purified µ-opioid receptor (or pertussis toxin-treated membranes) from rat brains, the A-kinase phosphorylation of receptor attenuated the µ-agonist-stimulation of low Km GTPase. These findings provide the evidence that µ-receptor possesses two different coupling sites involved in transducing the agonist signal to Gi and in increasing in high affinity agonist binding by coupling with Gi, and that the former is selectively inactivated by A-kinase.

423.7

OPIOID SENSITIVITY IN DIFFERENT STRAINS OF MICE CAN AFFECT OPIOID ANTAGONIST-INDUCED SUPERSENSITIVITY. B.C. Yoburn, S.P. Kreuscher*, V. Sierra*, M. Legatos* and S. Azimuddin*. College of Pharmacy, St. John's Univ., Queens, NY 11439.

Sensitivity to morphine's effects can vary with the strain of mouse employed. The ED_{50}s for morphine analgesia (tailflick) in Swiss-Webster mice from Charles River (CR) differed significantly from Taconic Swiss-Webster mice (T) and the C3H (Harlan SD) strain (ED_{50}s CR=6.2; T=3.8; C3H=2.5mg/kg). LD_{50}s in the Swiss-Webster strains differed significantly by more than 2-fold (LD_{50}s T=302; CR=707mg/kg). To determine if sensitivity to morphine affects the increase in opioid agonist potency produced by chronic naltrexone (NTX) treatment, mice were implanted subcutaneously for 8 days with NTX or placebo pellets. The pellets were removed and 24hr later ED_{50}s for morphine analgesia determined. NTX produced significant supersensitivity to morphine in the Swiss-Webster strains (morphine relative potencies for NTX-treated mice were T=2.0; CR=2.0). For the most sensitive strain, C3H, NTX produced a small, insignificant increase in potency (1.2). These data indicate that baseline sensitivity to morphine can determine the degree of the supersensitivity response, and raise the possibility that chronic opioid antagonist treatment produces minimal changes in opioid receptors in some morphine sensitive strains of mice. (supported by NIDA DA 04185)

423.8

BINDING SITES FOR THE ANTIOPIATE TYR-MIF-1 DECREASE AFTER CHRONIC MORPHINE. J.E. Zadina, A.J. Kastin, L.J.Ge*, H.Gulden, & K.Bungart*. VA Med.Ctr. & Tulane Univ. Sch. Med. New Orleans, LA 70146.

Opiate addiction could involve a change in the activity or binding of endogenous antiopiates. Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), which can antagonize exogenous and endogenous opiates (Life Sci.39:2153, 1986; Brain Res. 409:10,1987), could play such a role. Tyr-MIF-1 can bind to some opiate receptors with low affinity and to morphiceptin-labeled sites with high affinity, but its major action may be at its own high affinity binding site (Peptides 6:965,1985; Pharm. Biochem. Behav. 25:1303,1986). Rats given morphine (75mg) or placebo pellets (2 on day 1 and 4 on day 2) had reduced ^{125}I- and ^{3}H-Tyr-MIF-1 binding on day 5, when substantial tolerance and dependence are evident. In contrast, mu and delta opiate (^{3}H-DAGO, -DPDPE, or -DADLE labeled) receptors were increased. Acute injection of morphine (7.5 mg/kg), known to increase opiate receptors and analgesia, did not reduce Tyr-MIF-1 binding, indicating that chronic morphine administration is required to change Tyr-MIF-1 binding. The action of morphine on Tyr-MIF-1 binding sites could reflect a change in the activity of the tetrapeptide during the development of tolerance and dependence. These findings open new approaches to the study of addiction by focusing on antiopiate activity.

423.9

INOSITOLHEXAKISPHOSPHATE (PHYTIC ACID) ENHANCES $^{45}Ca^{2+}$ INFLUX AND ^{3}H-D-ASPARTATE RELEASE IN CULTURED CEREBELLAR NEURONS. P.L. Canonico, V. Bruno*, L. Fiore*, F. Nicoletti* and U. Scapagnini*. Institute of Pharmacology, Catania Univ. Sch. of Med., Catania, Italy.

Inositolhexakisphosphate ($InsP_6$), a putative metabolite of inositoltrisphosphate, may act as a neuromodulator in the CNS. $InsP_6$ accumulates in rat brain after in vivo labeling with ^{3}H-inositol and mimicks the action of glutamate when locally infused into the nucleus tractus solitarius (Vallejo, M. et al. Nature 330:656,1987). Here we report that addition of $InsP_6$ enhances $^{45}Ca^{2+}$ uptake and ^{3}H-D-aspartate release in cultured cerebellar granule cells, a homogeneous population of glutamatergic neurons. The action of $InsP_6$ is concentration-dependent, with an IC_{50} value in the low micromolar range. Stimulations of $^{45}Ca^{2+}$ uptake by glutamate and $InsP_6$ are additive, suggesting that $InsP_6$ does not bind to glutamate recognition sites. However, the stimulation of $^{45}Ca^{2+}$ influx by $InsP_6$ is attenuated in the presence of compounds which antagonize the action of endogenous glutamate at the Mg^{2+}-sensitive receptors. We suggest that $InsP_6$ potentiates glutamatergic transmission acting as a positive modulator at specific excitatory amino acid receptors in primary cultures of cerebellar neurons.

423.10

ACTIVATION OF PROTEIN KINASE C DECREASES GLUTAMATE SENSITIVITY OF CEREBELLAR PURKINJE CELLS. F. Crepel[X] and M. KRUPA[X](SPON:J. Hirsch). Lab.Neurobiologie et Neuropharmacologie du Développement. Université Paris-Sud, 91405 Orsay, France.

In the cerebellum, long term depression (LTD) of synaptic transmission between parallel fibres (PFs) and Purkinje cells (PCs) by concurrent activation of climbing fibres (CFs) is due to a selective desensitization of Quisqualate receptors of PCs to L-glutamate (Glu).

In the present experiments performed in 18 to 26 day old rat cerebellar slices in vitro, simultaneous activation of PCs by Glu and CFs led to a clear LTD of the responses of these neurones to Glu in 30% of the recorded cells while this effect was not observed for L-aspartate. This selective LTD of the sensitivity of PCs to Glu was also obtained in 22% of PCs by pairing Glu applications with calcium (Ca) spikes elicited by direct activation of the cells. Finally, selective LTD of Glu induced responses was obtained in all tested cells by bath application of 200 nM of phorbol esters known to activate protein kinase C (PKC).

This suggests that desensitization of Glu receptors during LTD is due to a coactivation of PKC by Ca and by diacylglycerol resulting from the stimulation of PCs through CFs and PFs respectively.

423.11

AMYGDALA KINDLING PRODUCES SPECIFIC DOWN-REGULATION OF HIPPOCAMPAL PHENCYCLIDINE RECEPTORS. R. Sircar, N. Ludvig*, S. L. Moshe* and S.R. Zukin. Departments of Psychiatry and Neuroscience, Neurology*, Albert Einstein College of Medicine, Bronx, NY 10461.

Phencyclidine (PCP) derivatives and drugs which elicit PCP-like behavioral effects, such as sigma opiates and dioxalanes, bind to specific PCP receptors. Recent findings suggest that PCP-like compounds mediate their neurobehavioral effects by non-competitive inhibition of brain N-methyl-D-aspartate (NMDA) receptors. D-(-)-2-amino-5-phosphonovaleric acid (D(-)APV), a highly selective NMDA receptor antagonist, abolishes the specific binding of PCP receptor radioligand N-(1-[2-thienyl]cyclohexyl)[^{3}H]piperidine ([^{3}H]TCP), suggesting that the PCP receptor may be localized to a site on the active conformation of a supramolecular PCP-NMDA receptor-ion channel complex. Neuroanatomical studies have demonstrated that the highest densities of both PCP and NMDA receptors are found in the CA_1 field of the hippocampus and the dentate gyrus. PCP and PCP-like drugs, including ketamine and the extremely potent PCP receptor ligand, MK-801, possess potent anticonvulsant properties. PCP raises the seizure threshold following amygdala kindling. Besides its direct applicability as a model of focal epilepsy ideal for the study of biochemical aspects of epileptogenesis, kindling has been used as a model of learning and synaptic plasticity. Here we report the effect of amygdala kindling on PCP receptor binding in specific rat brain regions. Rats were kindled from the left amygdala until they developed 3 consecutive stage 5 generalized seizures. Animals were sacrificed 72 hrs after the last stage 5 seizure. PCP receptor binding parameters were determined in specific brain areas. Among all the brain regions studied, consistent change was detected only in the hippocampus, where the density of PCP receptors was significantly decreased by 24.9% (B_{Max} = 3.31 pmol/mg protein versus 4.41 pmol/mg protein in sex-age matched naive animals). The apparent K_d values did not differ significantly between the experimental and control groups. Since the PCP receptor is an integral part of the NMDA receptor-ion channel complex, our results suggest that the NMDA receptor might be affected by kindling process, a concept also supported by others. PCP-NMDA receptor complex may play a critical role in the expression of kindling.

423.12

DIFFERENTIAL INTERACTIONS OF [^{3}H]2-OXO-QUAZEPAM BINDING WITH THE GABA RECEPTOR COMPLEX IN THE RAT BRAIN. R.T. McCabe[1], A. Barnett[2], L.C. Iorio*[2], and J.K. Wamsley[1]. [1]Dept of Psychiatry, U of UT Sch of Med, SLC, UT 84132 and [2]Schering-Plough Corp., Bloomfield, NJ 07003.

A metabolite of the hypnotic quazepam, 2-oxo-quazepam (2OXOQ), recognizes high-affinity benzodiazepine-1 (BZ_1) receptors. Interactions of subcomponents of the GABA complex are relevant physiological and pharmacological processes. Thus, [^{3}H]2OXOQ binding to BZ_1 receptors was investigated to further examine the functional significance of the association with the GABA complex. The binding of [^{3}H]2OXOQ to membranes was performed as previously described. Inhibition of [^{3}H]2OXOQ was examined with potencies in descending order: Rol5 1788, BCCE, CL 218,872, bicuculline, and picrotoxin. GABA, pentobarbital, and muscimol enhanced [^{3}H]2OXOQ binding. The presence of varying concentrations of bicuculline (10^{-3} to 10^{-9} M) did not entirely reverse the GABA and pentobarbital enhancement. Partial blockade of BZ_1 sites with BCCE or CL 218,872 eliminated the GABA and pentobarbital enhancement in the presence of variable concentrations of bicuculline. Additional analysis of BZ interactions with subcomponents of the GABA system also has been performed. These data provide greater evidence concerning the importance of the relationship of BZ sites with the GABA receptor complex and support previous studies of the heterogeneity of BZ receptor interactions.

REGENERATION: GENERAL II

424.1

STRUCTURAL RECOVERY IN AN INJURED CNS BY RADIATION TREATMENT. Z. Fuks*, A. Alfieri*, J.H. Kim*, and N. Kalderon¶ (SPON: J. Sparrow). The Rockefeller Univ.¶, and Dept. of Radiation Oncology, Memorial Sloan-Kettering Cancer Cnt., New York, NY 10021.

Injury of the CNS initiates a series of processes one of which is the formation of a scar that is composed primarily of reactive astrocytes. The objective of this study was to prevent glial scar formation by radiation therapy as the first step in an attempt to improve axonal regrowth and establishment of synaptic connectivity. Experiments were performed on the left olfactory bulb (OB) of rats, inflicting injury by surgical incision (top-to-bottom, cutting the projections of the mitral cells into the olfactory cortex). OBs were cryostat-sectioned and analysed by immunocytochemical and histological techniques. It was found that the low-dose rate γ-irradiation (80 cG/hr, total dose 20 Gy) of the injured CNS was effective in prevention of scar formation provided that it was given at 15-20 days postinjury. In OB samples which were severed and irradiated 16-18 days later, gliosis was reduced and partial structural continuity was restored along the site of incision and, in addition, up to 30% of the axotomized mitral cells population was rescued. In contrast, in the unirradiated samples, the severed OBs had degenerated, whereas at the site of incision in the partially cut OBs, cavitation was formed and at least 96% of the mitral cells frontal to the cut had vanished.

Supported by The Spinal Cord Research Foundation.

424.2

LOW-ENERGY HE-NE LASER IRRADIATION ENHANCES POTASSIUM-BUFFERING CAPABILITY OF GLIAL CELLS. I.E.Friedman, M.Belkin* and M.Schwartz, Dept. of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel.

Injury to a mammalian CNS axon usually leads to irreversible degeneration of the nerve. We have recently found that low-energy He-Ne laser irradiation causes a delay in degeneration of injured rat or rabbit optic nerves, provided that treatment is initiated immediately after injury (Rosner et al. submitted). We propose that this effect on the early stages of neural degeneration may be related to the early ionic changes which result directly from the injury, such as an increased level of extracellular K^+. Since glial cells are known to be involved in regulation of extracellular K^+, we decided to study, in vitro, the effect of low-energy laser irradiation on C-6 glioma cells.

We have found that irradiating C-6 glioma cell cultures for 1 minute with 3.15 mW from a He-Ne laser resulted in a 24% increase in the rate of $^{86}Rb^+$ uptake by the cells. This elevated rate increased to 8-fold for the 5 minute period follwing irradiation, with elevated levels being maintained for up to 1 hour.

The initial rate of $^{22}Na^+$ uptake decreased by 39%. Substituting choline for Na^+ reduced the laser induced increase in $^{86}Rb^+$ uptake, although this level was still higher than that of non-lasered controls. In the presence of 1 mM ouabain, irradiation still increased $^{86}Rb^+$ uptake. $^{45}Ca^{2+}$ uptake was not affected, but the extent of efflux from preloaded cells was reduced by 20% following laser treatment.

We propose that low energy He-Ne laser irradiation increases the K^+-buffering capablity of glial cells, which can then act to reduce the spreading degeneration of nerves following trauma.

Supported by grant 0355 from the National Council for Research and Development.

424.3

CONTRASTING NEUROGLIAL RESPONSES IN THE AXOTOMIZED HYPOGLOSSAL AND RED NUCLEI OF RAT. K.D. Barron, M.P. Dentinger and R. Amundson*. Depts. of Neurology and Neurosurgery, Alb. Med. Coll. and Research Svce. (Neurology) Vet. Adm. Med. Ctr., Albany, NY 12208, USA.

Within 1-3 days of facial neurotomy, hyperplasia of resident, resting microglia of the facial nucleus is demonstrable by lectin histochemistry (Streit and Kreutzberg, 1987) and protoplasmic astrocytes, normally inapparent in stains for glial fibrillary acidic protein (GFAP), become strongly immunoreactive (Graeber and Kreutzberg, 1986) and transform into hypertrophied fibrous astrocytes. We have confirmed these data for hypoglossal nucleus (HN) of rats killed 3, 14 and 28 days after 12th nerve section. Striking microglial hyperplasia was evident 3 days postoperatively and persisted through 28 days. Concomitantly GFAP-rich, reactive astroglia appeared. All animals were killed under anesthesia by intra-aortic perfusion with buffered formaldehyde followed by ethanol-acetic acid (3:1). Paraffin sections were cut at 4 μ. In the neurotomized rats, unilateral cervical rubrospinal tractotomy was done also. The axotomy response in the magnocellular division of the red nucleus (RN) contrasted with that in HN in that there were no histochemically-definable microglial or astrocytic changes. Additionally, in contrast to the HN, there was no increase in neuroglia in the axotomized RN while tractotomy did not cause a change in the cytochemically-quantifiable RNA of rubral neuroglia. Perineuronal oligodendroglia, immunohistochemically identified by transferrin content, appeared also to behave differently in HN and RN after axotomy. Hypoglossal neurons regenerate severed axons while the centrally projecting neurons of RN fail to do so. This failure may relate to a lack of neuroglial response similar to that observed around successfully regenerating cranial motoneurons.

Supported by the Veterans Administration.

424.4

PERSISTENCE OF THE MORPHOLOGICAL CHANGES INDUCED BY GM_1 IN AXOTOMIZED, COERULOSPINAL, NORADRENERGIC NEURONS: A CHRONIC STUDY. J.W. Commissiong and G. Toffano. Dept. of Physiol., McGill Univ., Montreal, Canada H3G 1Y6 and Fidia Res. Lab., 35031 Abano Terme, Italy.

Based on previous results (Brain Res. 380, 205-215, 1986; Soc. Neurosci. Abs. 13: 1509, 1987), the present studies were done to test whether the morphological changes induced by GM_1 in the axotomized, coerulospinal neurons, persist after termination of treatment with GM_1. Postnatal day 14 and 21 rats were anesthetized with ether and spinalized at the middle thoracic region. The animals were treated with GM_1 (30 mg/kg, i.p.) daily for the first 3 weeks, then every other day for the next 18 weeks. Treatment was then stopped for up to 18 weeks before the animals were processed. The changes induced by GM_1 in the axotomized, coerulospinal neurons: 1) increase in the number of somatic dendrites; 2) increase in the length of the somatic dendrites; 3) increase in the fluorescent intensity of the cells, persisted for up to 18 weeks after GM_1 treatment was discontinued. Of 240 cells analyzed in five GM_1-treated animals, 141 or 60% exhibited the morphological effects listed above. Of 95 cells analyzed in saline-treated rats, 8 or < 10% exhibited the morphological changes observed. The GM_1-induced morphological changes in the cord did not persist in the chronic phase.

424.5

REESTABLISHMENT OF CENTRAL PATHWAYS AND REINNERVATION OF THE PERIPHERY BY SEROTONERGIC NEURONS IN THE SNAIL MELAMPUS BIDENTATUS. S. B. Moffett and T. A. Howard*. Dept. of Zoology, Wash. State Univ., Pullman, WA 99164.

This study focused on regeneration of neurons and axons which exhibit serotonin-like immunofluorescence. Two lesions were performed: 1) a pedal nerve was crushed, or 2) a pedal ganglion was removed. Axons were disrupted at the crush site and proximal and distal portions of the axons showed retraction bulbs (1-2 days) followed by the formation of tiny projections from the proximal ends into the crushed region (3-5 days). Subsequent growth of the proximal ends into the periphery kept pace with proximal-to-distal disintegration of distal processes (6-12 days).

A ganglion bud consists of some or all of the following: unification of the cerebropedal and pleuropedal connectives (5-7 days), growth between the intact pedal ganglion and the connectives (12-14 days), and regrowth of pedal nerves (12-21 days). Serotonergic neurons in the intact pedal ganglion, the parietal and visceral ganglia and the cerebral ganglia contribute to bud and pedal nerve formation. If the new growth fails to unite with the distal nerve stump, the distal serotonergic staining disappears (7-12 days); longer-term regeneration (1-6 mo) reveals highly variable serotonergic reinnervation. In some cases, identified cerebral neurons known to project to the missing pedal ganglion lose serotonergic immunofluorescence after pedal ganglion removal. (Supported by NIH RO1 NS 22896 to S.B.M.)

424.6

REGENERATION OF SEROTONERGIC AXONS PROJECTING TO THE PENIAL COMPLEX IN THE SALT MARSH PULMONATE SNAIL MELAMPUS. R.L. Ridgway, R.M. Bailey*, and S.B. Moffett. Dept. of Zoology, Washington State Univ., Pullman, WA 99164-4220.

The penial complex (vas deferens, penis, preputium) of Melampus bidentatus is innervated by an unpaired penial nerve that arises from the right cerebral ganglion (RCeG). Backfilling of this nerve with nickel chloride shows two clusters of small neurons: one in the RCeG (25-30 cells), the other in the right pedal ganglion (RPeG) (10-15 cells). The RPeG cluster exhibits serotonin-like immunoreactivity (SLIR). The processes of these cells project to the RCeG via the right cerebropedal connective, exit the RCeG via the penial nerve, and then terminate in the muscle layers of the penial complex. We examined the ability of these serotonergic cells to reinnervate the penial complex after RCeG removal. Distal axon stumps and terminal branches show evidence of degeneration within 3 days of lesioning and SLIR usually disappears from the penial complex by 7 days. Over the same period, regenerative growth is both rapid and specific. Proximal axon stumps sprout in the first 3 days after ganglionectomy and grow toward the penial nerve stump, reaching the penial complex in 10-15 days; in some regions SLIR approaches that of control specimens by 30 days after lesioning. Axonal growth cones of the serotonergic cells do not appear to fuse with distal axon stumps and their pattern of growth is not restricted to that of the original axons. (Funded in part by NIH RO1 NS 22896 to S.B.M.)

424.7

EARLY CHANGES IN PUTATIVE NUCLEAR PROTEINS FOLLOWING AXOTOMY. A. Buriani, M.J. Savage and D.J. Goldberg. Dept. of Pharmacology, Columbia U. Coll. of P&S, New York, NY 10032.

In the days following axotomy, neurons alter their synthesis of protein; regenerating neurons usually greatly increase the synthesis of a few proteins (GAPs), for example. Such effects are likely to be caused by early changes in the abundance or activity of certain nuclear proteins. We have thus examined early (i.e., 4-5 hours post-axotomy) changes in the incorporation of ^{35}S-methionine into nuclear proteins. Nuclei were obtained from R2 neurons of *Aplysia californica*. There were two main advantages in using this system: first, we obtained neuronal nuclei only and, second, we could clean nuclei individually under the dissecting microscope. Two-dimensional SDS-PAGE was then performed on the labeled proteins and a quantitative analysis demonstrated significant changes in the labeling of four proteins. One (m.w. 56 kD; pI 5.8) showed a 15x increase and another (41 kD; 5.3) a 9x increase. Two other proteins incorporated less label, one (47 kD; 5.2) showing a 4x and the other (77 kD; 6.0) a 2x decrease. We are now determining whether there are similarly early changes in phosphorylation of these or other putative nuclear proteins. Their probable nuclear location and rapid response to axotomy make these 4 proteins candidate factors in the triggering of later changes in protein synthesis.

424.8

CONDITIONED MEDIA OF REGENERATING FISHOPTIC NERVES MODULATE LAMININ LEVELS IN GLIAL CELLS. Cohen A.* and Schwartz M. The Weizmann Institute of Science, Dep. of Neurobiology, Rehovot, Israel.

The absence of laminin in the central nervous system of adult mammals might be a reason for their poor regenerative ability. Previously, we have shown that medium conditioned (CM) by regenerating fish optic nerves, applied *in situ* to injured optic nerves of adult rabbit, causes an increased appearance of laminin immunoreactive sites (Zak. et al. Brain Res. 408: 263 ,1987). This observetion suggests that CM can modulate laminin levels in glial cells. In the present study, we show that this CM can activate C-6-glioma cells to synthesize and accumulate laminin. The level of laminin immunoreactive sites was elevated in cells treated with CM, as revealed either by ELISA screening method for surface antigen or by immunofluorecence, by using laminin specific antibodies.The optimal effect was observed at concentration of 0.1 *ug/ml* protein and was even higher than the effect of 10% fetal calf serum. The identity of the laminin immunoreactive protein and the observed effect of CM on C-6-glioma cells was verified by metabolic labelling of these cells, followed by immunoprecipitation and gel-electrophoresis. The CM-induced effect was not unique to laminin as a similar elevetion could be observed in fibronectin level (another matrix protein). This CM-induced effect could be detected in the pool of high salt extractable proteins as well as in the medium. Boiling of the CM completely abolished it's activity, suggesting that a proteinaceous component(s) within the CM is responsible for the activation. Production of laminin may be a necessary step in the induction of regeneration. Therefor, application to injured mammalian CNS, of factor(s) originating from regenerating systems, that can modulate laminin production or accumulation in glial cells may circumvent one of the impediments to regeneration.

424.9

INCREASED TRANSFERRIN RECEPTOR EXPRESSION BY REGENERATING RAT FACIAL MOTOR NEURONS. M.B.Graeber*, G.Raivich* and G.W.Kreutzberg Dept. of Neuromorphology, Max Planck Institute for Psychiatry, Am Klopferspitz 18a, D-8033 Martinsried, F.R.Germany.

The distribution and time course of transferrin receptor expression were studied in normal and regenerating rat facial nuclei following cut lesion in the facial nerve.
1. Immunocytochemistry (Ox-26 monoclonal antibody) revealed a transient increase (days 2-14) in transferrin receptor expression on regenerating facial motor neurons reaching a peak by day 5 after operation. 2. This transferrin receptor immunoreactivity was absent from resting (unoperated facial nucleus) and reactive (axotomized facial nucleus) astrocytes and microglial cells. 3. ^{125}I-transferrin binding studies showed an approximately threefold increase in transferrin binding sites confined to the regenerating nucleus and with the same time course as observed by immunocytochemistry.
In summary, there is a dramatic increase in trans ferrin receptors on regenerating motor neurons. We suggest that this elevated capacity to take up transferrin and iron may play an important role for neuronal repair. (SPON: W. Streit)

424.10

MYELIN DEFICIENT MUTATION AS A TOOL FOR STUDYING THE NON-PERMISSIVE NATURE OF CNS REGENERATION. I.J. Kljavin, N.S. Dobratz*, A.J. Jacobs and R. Madison. Depts. of Neuropathology and Neuroscience, Harvard Medical School and Children's Hospital, Boston, MA 02115.

Regrowth of axons in the central nervous system (CNS) may be limited by the absence of necessary molecules supporting nerve growth and/or the presence of inhibitory molecules found in CNS myelin (Caroni & Schwab., Neuron., 1:85,1988). Growth of dorsal root ganglion (DRG) explants and dissociated cells was assessed on culture substrates taken from the CNS (optic nerve) and PNS (sciatic nerve), of wild type C57BL6 mice and shi/shi (an outbred stock). Shiverer is a hypomyelinated mutant lacking proteolipid and basic myelin proteins.

Cryostat sections of optic and sciatic nerves 6um thick were placed onto polylysine coated glass slides. Neuronal cultures of DRG explants and dissociated cells were prepared from E19 mouse embryos, plated onto the tissue substrates, maintained in DMEM+FCS+NGF, and examined by light and scanning electron microscopy. DRG's grew extensive fibers (more than 2X ganglion diameter; up to 10X cell diameter) onto sciatic nerves. Nerve fiber growth from explants onto normal optic nerve was rare, but a few dissociated DRG's showed limited growth (up to 3X cell diameter). Shiverer optic nerves supported more neuronal growth (at least 1X ganglion diameter; up to 5X cell diameter) but also supported noticably more non-neuronal growth compared to normal optic nerve. The potential influence of non-neuronal cells on nerve fiber outgrowth must be considered with this culture system. Supported by NS22404 to RM.

425.1

HOMOGRAFTED FETAL RAT CORTICAL ASTROCYTES MIGRATE FROM CORTICAL IMPLANTATION POCKETS THROUGHOUT ADULT HOST RAT BRAIN W.J. Goldberg and J.J. Bernstein. Laboratory of CNS Injury and Regeneration, VA Medical Center; Departments of Neurological Surgery and Physiology, George Washington University School Medicine, Washington, DC.

The cerebral cortices from 14 day gestation rat embryos were prelabeled with the lectin *Phaseolus vulgaris* leucoagglutin (PHAL) and homografted into freshly made implantation pockets in host cerebral cortex. Animals were used 30 and 60 days later. Paraffin sections were double labeled for glial fibrillary acidic protein (GFAP, astrocyte specific marker), and PHAL (graft specific marker). A PHAL-GFAP positive cell was a graft derived astrocyte. Graft derived astrocytes were found on glia limitans along the entire circumference of the brain, in the hippocampal commissure, corpus callosum, internal capsule, entopeduncular nucleus, habenular commissure, brachium of the superior colliculus, optic tract, optic chiasm and sensory root of the trigeminal nerve. Grafted astrocytes entered the spaces of Virchow-Robin, migrated along parenchymal blood vessels and between the ependymal and subependymal layers of the third and lateral ventricles. Graft derived astrocytes migrated ventrally through the gray matter at the base of the implantation pocket to enter the corpus callosum. The migration routes continued through intersecting nerve fiber bundles. Basal lamina (blood vessels, glia limitans, space between ependymal and subependymal layer) was another preferred migration route. Since a major constituent of basal lamina is laminin, these data suggest that laminin may be one of the cell surface recognition molecules for fetal astrocyte migration.

Supported by the Veterans Administration.

425.2

EFFECTS OF ARACHNOID-PIAL CELLS ON IN VITRO ASTROCYTIC GAP JUNCTIONAL COMMUNICATION MEASURED BY FLUORESCENCE RECOVERY AFTER LASER PHOTOBLEACHING. J.J. Anders. Anatomy Department, USUHS, Bethesda, MD 20814

Gap junctions are prevalent between astrocytic processes of the glia limitans. An interaction between astrocytes and arachnoid-pial cells may establish a regional specialization in the transfer of ions and small molecules by astrocytes at this important CSF-brain interface. The purpose of this study is to test the effect of the presence of arachnoid-pial cells on gap junctional conductance between astrocytes. A slide of rat arachnoid-pial cells was sandwiched against a glass slide on which primary rat astrocytes were grown. A 1mm thick rubber gasket separated the slides. The cells were cocultured for up to 72 hrs. The arachnoid-pial cells were removed and astrocytic gap junctional communication was examined. Fluorescent molecules (carboxyfluorescein) in the astrocytes were bleached with an argon ion laser. Fluorescence recovery due to gap junctional mediated diffusion of unbleached molecules from adjacent cells was monitored. The mean total recovery of fluorescence for control astrocytes was 25%. Astrocytes cocultured with arachnoid-pial cells for 48 hrs did not show a difference in the recovery of fluorescence. However, after 72 hrs of coculture, the mean total recovery of fluorescence increased to 62% ($P<0.001$ on T-test). These results indicate that the presence of arachnoid-pial cells increases astrocytic gap juctional conductance.

425.3

THE MOLECULAR FORMS OF PLASMINOGEN ACTIVATOR IN DIFFERENTIATING ASTROGLIA ARE DEVELOPMENTALLY REGULATED. N. Kalderon. The Rockefeller Univ., New York, NY 10021

Plasminogen activator (PA) is the key enzyme which initiates a cascade of extracellular proteolytic activities. Mammalian cells produce two distinct molecular forms of PA, the tissue-type -- t-PA and the urokinase-type -- u-PA. Recent studies show that the role of u-PA is in control of cell migration/invasion, while t-PA is involved primarily in fibrinolysis. It was established that the cellular PA activity levels of differentiating rodent astroglia are developmentally regulated (Kalderon et al., 1988 in Cur. Issues in Neural Regen. Res., Reier et al., eds., in press). This study focuses on the characterization of the molecular forms of PA which are produced by differentiating rat astroglia in cell culture. It was found that: 1) immature glial cells until the age corresponding to postnatal day 10 (P10) express mostly the u-PA form (M_r ~47K), 2) purified astroglia at cell ages P12-P24 produce both u-PA and t-PA forms, and 3) astrocytes at cell age P30 and/or older produce only the t-PA form (M_r ~81K). Since no PA activity was detected in purified oligodendroglia (P11) it is assumed that the glial PA activity is primarily of astroglia. It is concluded that the expression of PA molecular forms in astroglia is developmentally regulated. u-PA is the predominant form in the immature astrocyte. With cell maturation u-PA disappears while the t-PA type is being produced, and in the mature astrocyte t-PA seems to be the sole form.

Supported by NIH (NS23064).

425.4

ION CHANNELS IN RAT ASTROCYTES IN PRIMARY CELL CULTURE. C.L. Bowman *and* M. Sokabe*, *Department of Biophysical Sciences. SUNY - Buffalo, Buffalo, NY 14214.*

Using the single-channel recording technique in the cell-attached configuration, we are investigating several channels that exist in astrocytes (a type of glial cell) derived from neonatal rat brains. With a Mg-free Ringer's solution in both the bath and pipette, we observe several classes of channels. The first and most common (15 out of 22 cells) class consists of brief events lasting several msecs composed of several amplitudes. At depolarizing membrane potentials, the P_o and amplitude increases suggesting that this class of channel is either Cl or K selective. The second class of channel has a larger amplitude and a very long open duration (seconds) (9 of 22 cells). The third class of channel consists of spiky brief events, that occur in bursts at hyperpolarizing membrane potentials (5 of 22 cells). The fourth and final class of channel is a slow gating (10's of msecs) small amplitude event (2 of 22 cells).

With a 200 micromolar glutamate Ringer's solution in the pipette, the frequency of appearance of the slow gating channel increases (8 of 16 cells) and the spiky bursting events become observable at -60 mV, the normal membrane potential (5 of 16 cells). The reversal potential of the latter class of channel is near 0 mV.

We thank Drs. Malcolm Broderick and Mike Curran for useful suggestions. Supported by NIH grants R23 NS-24891 to CLB, Nitto Foundation to MS, and USPHS DK 37792 and USARO 22560 - LS to Frederick Sachs.

425.5

A STRETCH-ACTIVATED ION CHANNEL IN RAT ASTROCYTES IN PRIMARY CELL CULTURE. J-P.Ding*, X-C.Yang*, C.L.Bowman, and F. Sachs* (SPON: A. Auerbach). Department of Biophysical Sciences, SUNY - Buffalo, NY 14214.

Using single-channel patch-clamp recording, we have observed a stretch-activated channel (SAC) in rat astrocytes. The channel can be activated in both cell attached and inside-out excised patches and opens with 1-5 cm Hg suction applied to the pipette. The channel is present in more that 50% of the cells tested (ca. 100 cells), and is most easily observed with KCl filled pipettes. With 110 mM KCl in the pipette, the single channel conductance is about 40 pS and the I/V curve is linear, reversing at a membrane potential of approximately -60 mV. The probability of being open (Po) is voltage dependent and at 4 cm Hg ranges from 0.005 at -60 mV to 0.02 at +10 mV. The channel is blocked by 1 mM extracellular CsCl and partial blockage is visible at 10 micromolar. With NaCl or CsCl filled pipettes, an outward channel current become observable at membrane potentials more positive than +20 mV. These results suggest that the channel is K selective. The probability of the channel being open is linearly related to the square of the applied suction suggesting that gating energy is derived from elastic deformation of the channel as proposed by Guharay and Sachs (J.Physiol. 352:685, 1984) for SACs in chick muscle.

In-vivo, this channel may play a role in volume regulation of the glial cell and in the spatial buffering of potassium. Since the membrane is K permeable, elevation of extracellular K would cause the cell to swell. Swelling would open more K selective SACs thereby increasing the rate of K uptake and lowering the local extracellular levels of potassium.

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425.6

EFFECT OF NIMODIPINE ON POTASSIUM (K^+) STIMULATED CALCIUM (Ca^{++}) UPTAKE IN ASTROCYTES. H.S. White*, A.S. Bender*, D.M. Woodbury, and L. Hertz*[1]. Dept. of Pharmacol. and Tox., Univ. of Utah, S.L.C., UT 84112 and [1]Dept. of Pharmacol., Univ. of Saskatchewan, Saskatoon, Canada S7N 0W0.

Previous attempts to demonstrate that K^+ stimulates $^{45}Ca^{++}$ uptake into astrocytes have been unsuccessful. Primary astrocytic cultures were incubated with serum-free media containing $^{45}Ca^{++}$ (0.5 μCi/ml) and K^+ (5.4 to 120 mM) for varying lengths of time (15 sec to 30 min). Uptake was terminated by 5 washes with 0.32 M (0-2°C) sucrose containing 2.0 mM EGTA. $^{45}Ca^{++}$ uptake was time-dependent and reached a peak steady-state level of 6 to 7 nmol/mg protein within 10 min. No increase in the 90-sec $^{45}Ca^{++}$ uptake was observed in cells simultaneously incubated with $^{45}Ca^{++}$ and depolarized with 50 mM K^+. In contrast, $^{45}Ca^{++}$ uptake into cells first loaded with $^{45}Ca^{++}$ for 60 sec and then depolarized with 50 mM K^+ for an additional 30 sec was increased 61% above unstimulated controls. This effect of K^+ was completely attenuated by the Ca^{++} channel blocker nimodipine (IC50: ≈2 nM). High concentrations (0.1 to 100 μM) of nimodipine only partially (20-30%) reduced basal uptake. Thus, under optimal conditions, $^{45}Ca^{++}$ uptake into primary astrocytes is enhanced by K^+. That nimodipine blocks this effect suggests that the utility of Ca^{++} blockers in ischemia and epilepsy may be related in part to an effect on astrocytes.

(Supported by NIH Grant 1-RO1-NS 22200 and MRC Grant MT5957).

425.7

POTENT MODULATION OF POTASSIUM (K^+) UPTAKE INTO ASTROCYTES BY PROTEIN KINASE C (PKC). A.S. Bender*, L. Hertz*[1], D.M. Woodbury, and H.S. White* (SPON: J.U. Wei). Dept. Pharmacol. and Tox., U. of Utah, S.L.C., UT 84112 and [1]Dept. Pharmacol., U. of Sask., Saskatoon, Canada S7N OWO.

Within the CNS, astrocytes play an important role in the regulation of extracellular K^+. The mechanisms of this process are complex and include both active uptake and spatial buffering. The present investigation was initiated in order to assess the effect of phorbol esters on active $^{42}K^+$ uptake and passive $^{42}K^+$ permeability of cultured mouse cerebral cortical astrocytes according to the method of Hertz (Ann. N.Y. Acad. Sci. 481:318-333, 1986). The active phorbol ester 12-myristate 13 acetate (PMA) inhibited both active uptake of $^{42}K^+$ and passive $^{42}K^+$ permeability. Its effect on passive permeability was, however, significantly greater than its effect on active transport (IC50: ≈0.07 vs 81 μM). In contrast, the inactive congener 4α-phorbol 12,13-didecanoate did not exert any effect on K^+ permeability at concentrations up to 1 μM. Since it is widely recognized that phorbol esters activate PKC, the present results suggest that this enzyme plays an important role in modulating passive K^+ permeability into astrocytes. This is a novel mechanism whereby passive K^+ influx into astrocytes is regulated and may ultimately play a role in the regulation of CNS excitability. (Supported by NIH Grant 1-RO1-22000 and MRC Grant MT5957).

425.8

Intracellular pH regulation in cultured mammalian astrocytes. G. Boyarsky*, B.R. Ransom, W.G. Carlini, W.F. Boron* Depts. of Physiol. and Neurology. Yale Univ., Sch. of Med., New Haven, CT 06510.

Intracellular pH (pH_i) regulation was studied in single cultured rat astrocytes. pH_i was measured using the fluorescence excitation spectrum of the dye BCECF, and calibrated using the high K^+/nigericin technique. A 10-μm spot of light was focused on individual cells on an epifluorescence microscope. Cells were continuously superfused at 37°C. In HCO_3^--free, HEPES-buffered solution, the steady-state pH_i was ~6.9. Removal of external Na^+ caused an abrupt acidification. Rapid recovery to near initial pH_i followed the readdition of Na^+. Switching from a HEPES- to a HCO_3^-- buffered solution resulted in a rapid alkalinization of ~.2 pH. This rapid alkalinization was blocked by removal of external Na^+, inhibited by removal of external Cl^- (replaced by cyclamate$^-$),and partially inhibited by pretreatment with SITS, an inhibitor of HCO_3^- transport in other cells. In some experiments, removal of Cl^- in the presence of HCO_3^- elicited an alkalinization consistent with $Cl^-HCO_3^-$ exchange activity. Increasing external K^+ from 5 to 25 mM, which depolarizes these cells by ~30 mV, caused an abrupt and reversible alkalinization of ~.2 pH, both in the presence and in the absence of HCO_3^-. We have demonstrated that mammalian astrocytes, cells which have long been thought to play an important role in pH regulation in the brain, actively regulated their pH_i by an acid-extrusion process(es)that is dependent upon Na^+, HCO_3^- and Cl^-

425.9

IDENTIFICATION OF GLUCOCORTICOID REGULATED PROTEINS IN PURIFIED RAT CEREBRAL ASTROCYTES BY QUANTITATIVE 2D-GEL ELECTROPHORESIS. M. C. Bohn, A. Walencewicz*, M. Lynch* and J. deVellis Department of Neurobiology and Anatomy, Univ. of Rochester Medical Ctr., Rochester, NY 14642 and Mental Retardation Research Ctr. Univ. of California, Los Angeles, CA 90024.

Although glucocorticoids are known to act in target cells to regulate gene expression at the transcriptional level, only a few glucocorticoid regulated genes have been identified in the nervous system. Previous studies have demonstrated that glutamine synthetase is regulated at the transcriptional level in astrocytes. This study was undertaken to identify other glucocorticoid regulated proteins in astrocytes.

Astrocytes were prepared from newborn rat cerebral hemisphere and grown in DME-F12 with 10% fetal calf serum. After 2 weeks, oligodendrocytes were removed by shaking, and the astrocytes replated and grown to confluency. On day 37, the cells were shifted to serum-free medium and, a day later, 10^{-6}M hydrocortisone (HC) added for 48 hours. During the last 16 hours, cells were labeled with ^{35}S-methionine (1mCi/dish) in methionine-free medium in the presence of HC. Control cultures were run in parallel. High resolution 2D gels were run by Protein Databases, Inc (n=3).

600 and 1000 cellular proteins on 6-8, 10% and 3-10, 12.5% 2-D gels, respectively, were subjected to quantitative computer analysis. Approximately 5% of the proteins were significantly increased or decreased by HC, suggesting that protein metabolism in astrocytes is markedly affected by glucocorticoids. These data promise to lead to the identification of glucocorticoid regulated genes in the brain.

Supported by NIH grants NS20832 and HD 06576.

425.10

ASTROCYTES AS A SOURCE OF PROSTANOIDS IN THE CNS: RELEASE OF THROMBOXANE EVOKED BY ATP. S.Murphy* and B.Pearce* (SPON: W. Steele).Biology Dept., Open University, Milton Keynes MK7 6AA, England.

Using primary cultures of cells from neonatal rat brain we have shown that astrocytes can synthesise and release thromboxane (TX) and prostaglandins PGE_2 and $PGF_{2\alpha}$ (Murphy,S. et al.,Neurosci. Lett.,61:61,1985;Pearce,B. et al.,FEBS Lett.,211:73,1987;Jeremy,J. et al.,Brain Res.,419 364,1987). However, apart from calcium ionophores and phorbol esters, we have been unable to find a physiological stimulus for prostanoid release (Murphy,S. and Pearce,B. Prost.Leuk.Ess.Fatty Acids Rev.,1:1,1988) until now. Activation of P2-purinergic receptors on vessels by ATP prompts the release of prostanoids. In our astrocytes, ATP and ADP (EC_{50}=50uM), but not AMP nor adenosine, evoke TX release within 1 min, and this involves the activation of phospholipase A_2(PLA_2).This action of ATP and ADP is mediated by P2 receptors linked to the hydrolysis of inositol phospholipids and the mobilisation of intracellular calcium. When cells are calcium-depleted and then stimulated with ATP, there is a marked delay in TX release associated with the time taken for the calcium pool to re-fill.

This work is in collaboration with James Jeremy and Paresh Dandona (Royal Free Hospital,London), and is supported by a project grant to SM from the SERC. The new permanent address for SM: Pharmacology, Univ. Iowa, Iowa City IA52242

425.11

SUBSTANCE P IMMUNOREACTIVE ASTROCYTES IN MULTIPLE SCLEROSIS PLAQUES. S.K. Kostyk, N.W. Kowall, S.L. Hauser* Neurology Service, Massachusetts General Hospital, Boston, MA 02114

The biology underlying the formation of demyelinated plaques in multiple sclerosis (MS) is not well understood. Recent studies have suggested the involvement of an autoimmune process. The neuropeptide, substance P, has been implicated in the inflammation of autoimmune rheumatoid arthritis. Substance P innervation of cerebral vessels and perivascular substance P immunoreactive astrocytes have been observed in normal tissue (Michel et al., Brain Res. 377:383, 1986). Using immunoperoxidase methods with well characterized rabbit polyclonal substance P antibody (INCstar), we examined human post-mortem brain tissue for substance P immunoreactivity in MS plaques. Preadsorbed antisera did not produce specific staining. Prominently stained astrocytes were found near the edge of the MS plaque often in association with small blood vessels. Many of the astrocytes were binucleate. Fewer substance P immunoreactive astrocytes were observed in the remainder of the plaque or surrounding tissue. These data suggest involvement of substance P immunoreactive astrocytes in the genesis of MS plaques.

425.12

TROPOMYOSIN ISOFORM EXPRESSION IN NORMAL AND NEOPLASTIC ASTROCYTES, P.G. Galloway, M.J. Likavec*, G. Perry, Case Western Reserve University, Cleveland, Ohio 44106

Tropomyosin is a protein associated with microfilaments of nonmuscle cells, having molecular weights from 29kD to 40kD. Changes in the expression of tropomyosin isoforms have been reported in transformed cells (J Biol Chem 258: 1983). We have examined tropomyosin isoform expression in human astrocytomas by immunostaining brain tumor sections and paper blots from brain tumor homogenates subjected to SDS-PAGE. Controls were normal white matter and white matter with reactive astrocytes. The tropomyosin antibodies used are listed as follows by tissue of origin and isoform recognition: platelet, 30, 32kD (PT); brain, 30, 35kD (BT); smooth muscle, 32, 36, 40kD (SM1) and smooth muscle, 30 and 32kD (SM2). Immunoreactivity of astrocytes in normal brain was present, but less intense than in neoplastic cells regardless of antibody. Immunoreactivity using BT was stronger than for PT for all degrees of anaplasia of tumors. This suggests that the 35kD isoform may be synthesized to a greater extent than the 30kD isoform in neoplastic cells. The immunoreactivity with PT and SM2 was maximal in the cytoplasm, whereas with BT and SM1 it was in cytoplasm and processes. Cytoplasmic staining by PT was smooth, but with BT it was granular. These data suggest that the different isoforms may have specific subcellular localization and organization in neoplastic cells. (Supported by CWRU Cancer Center Grant #P30CA43703 and CCHF Grant to PGG.)

426.1

TRANSMITTER CONTENT AND AFFERENT CONNECTIONS OF PROGESTERONE RECEPTOR (PR) CONTAINING NEURONS IN THE PRIMATE HYPOTHALAMUS Leranth, C.[1,2], MacLusky, N. J.[1,2], Redmond, D. E.[3] and Naftolin, F.[1]*Yale U. Medical School, Depts. of Ob/Gyn.[1], Neuroanatomy[2] and Psychiatry[3].

The aims of this study were to determine [A] the anatomical location and transmitter content of PR containing neurons in the primate hypothalamus; and [B] the afferent connections to these neurons from other hypothalamic transmitter systems. Single- and double immunostaining techniques were employed in the hypothalamus of ovariectomized (OVX) and OVX - estrogen primed (estradiol valerate, EV; 4 days, 20mg/day, i.m.) adult Green monkeys (*Cercopithecus aethiops*), using antibodies directed against PR, glutamic acid decarboxylase (GAD), ACTH, tyrosine hydroxylase (TH) and 5-HT. Results: A dense population of PR immunoreactive neurons was observed in the hypothalamic infundibular and ventromedial (VM) nuclei, and in area between the VM nucleus and the fornix. PR immunostaining was confined to the cell nucleus and increased markedly in intensity after EV treatment. Staining was unaffected by injection of progesterone (4mg, s.c. 2h before sacrifice). In colchicine pretreated monkeys (560µg, icv. 48h prior to sacrifice), the perikarya of all PR immunoreactive neurons were immunoreactive for GAD, although the majority of GAD immunoreactive neurons did not exhibit PR staining. In non-colchicine pretreated animals, GAD, ACTH, TH, and 5-HT immunoreactive synaptic terminals were identified on PR-reactive cells. Conclusions: These results suggest that in the monkey 1) a subpopulation of the hypothalamic GAD immunoreactive (presumably GABAergic) neurons is estrogen-sensitive and represents the primary target for the direct genomic actions of circulating progesterone; 2) the activity of GABAergic PR containing neurons may be influenced by input from other neurons containing GABA, catecholamines, opiocortins and 5-HT. (Supported by NIH-HD135867 and St. Kitts Biomed. Res. Foundation).

426.2

TRANSMITTER CONTENT AND AFFERENT CONNECTIONS OF PROGESTERONE RECEPTOR (PR) CONTAINING NEURONS IN THE GUINEA-PIG HYPOTHALAMUS N.J. MacLusky, C. Leranth, T.J. Brown and F. Naftolin*, Dept. of Ob/Gyn, Yale University Medical School, New Haven CT06510.

The nature of the hypothalamic systems controlling female reproductive cyclicity remains poorly understood. In this study, we have examined the afferent innervation and phenotypic characteristics of PR containing neurons in the guinea-pig hypothalamus. Light and electron microscopic studies were performed using antibodies directed against PR, glutamic acid decarboxylase (GAD), ACTH, tyrosine hydroxylase (TH) and 5-HT. RESULTS: In OVX animals, PR immunoreactivity was not detectable. After estrogen priming, PR immunopositive cells were observed in the hypothalamic arcuate and ventromedial nuclei, and in the periventricular and medial preoptic area. PR immunostaining was confined to the cell nucleus, primarily associated with the dispersed euchromatin. The nucleolus remained unstained. In non-colchicine pretreated animals, numerous GAD, ACTH, TH, and 5-HT immunoreactive synaptic terminals were identified in contact with the PR-reactive cells. Colocalization of PR with these antigens in the same cell was not observed. After colchicine pretreatment (100µg, icv. 32h prior to sacrifice), however, cytoplasmic immunoreactivity for GAD was observed in the majority of PR immunopositive cells. CONCLUSIONS: These results suggest that in the guinea-pig a subpopulation of hypothalamic GABAergic neurons is sensitive to estrogen and progesterone. These GABAergic PR-positive neurons may act as integrators for synaptic input from a number of other systems, including other GABAergic neurons, as well as neurons containing catecholamines, proopiomelanocortin-derived peptides and 5-HT. (Supported by NIH grant No. HD135867).

426.3

Control of Tyrosine Hydroxylase in Sympathetic Ganglia by Testosterone. M.E. Goldstein, A.W. Tank and R.W. Hamill. Dept. of Neurology, University of Rochester/Monroe Community Hospital, Rochester, NY 14603.

The hypogastric ganglion (HG) of the rat sympathetic nervous system is dependent upon the continued presence of testosterone for normal development and maintenance of tyrosine hydroxylase (TH) activity. Previous studies reveal a progressive decrease in TH activity in HG of adult male rats 1, 2 and 4 weeks following castration, and replacement of testosterone immediately following castration prevents this decrease. The regulation of TH by testosterone has been examined further to determine whether changes in TH activity are a direct result of decreased mRNA and protein levels or a change in the level of activity of preexisting enzyme molecules. Dot blots of total RNA isolated from HG hybridized with a cDNA probe coding for TH reveal a decrease in TH-specific mRNA following castration. To determine changes in TH protein in HG in response to castration, increasing concentrations of TH antiserum has been incubated with homogenates of HG, and following centrifugation, TH activity in the supernatants was measured to determine what concentration of antiserum is required to remove 50% of the TH activity. TH protein decreases in a graded fashion following castration. The changes in TH mRNA and protein follow the decrease in TH activity suggesting that testosterone regulates TH at the level of transcription.

426.4

PREGNANCY-INDUCED ALTERATION OF GABA-A RECEPTOR SENSITIVITY IN THE BRAIN: AN ANTECEDENT OF POST-PARTUM BLUES? M.D. Majewska and G. Falkay*. NIDA, Addiction Research Center, Baltimore, MD 21224, and Dept. of Obst. and Gynecol., Univ. Med. School, Szeged, Hungary.

The GABA-benzodiazepine-chloride ionophore (GABA-A) receptor complex in the brain is regulated by several endogenous steroids, including tetrahydroprogesterone (THP); (Majewska, M. D., Biochem. Pharmacol., 36:3781 1987). Pregnancy is associated with greatly elevated levels of progesterone, whose metabolites may affect the function of the CNS.

We compared the binding of the GABA-A agonist, [^{3}H]-muscimol, in vitro, in brain synaptosomes from non-pregnant rats and those at various stages of pregnancy. At 10 nM ligand concentration, there was about 50% and 30% more muscimol binding on days 15 and 19 of pregnancy, respectively, as compared to non-pregnant rats. This effect was due to a reduction of the Kd of low affinity GABA-A receptor sites. The alterations of muscimol binding caused by pregnancy were similar to the effects of THP, in vitro, and followed the pattern of changes of progesterone levels in the plasma of pregnant rats. It is likely that these pregnancy-induced changes resulted from increased levels of GABA-A-agonistic progesterone metabolites in the CNS.

The results may have impact on the behavioral and psychological changes associated with pregnancy and post-partum period.

426.5

IDENTIFICATION OF A BRAIN SITE FOR THE ACTION OF MELATONIN IN THE FEMALE MONGOLIAN GERBIL (Meriones unguiculatus). S.A. Ferreira[s], M. DeVries[s] and J.D. Glass[s] (SPON: J. Walro) Dept. Biological Sciences, Kent State Univ., Kent, OH 44242.

The pineal, via its hormone melatonin, is the primary transducer of photic information for neuroendocrine regulation of seasonal breeding. The site of melatonin action in the brain is uncertain; however, studies in mice have implicated the anterior hypothalamus (AH). The present study was undertaken to examine this site in another species. In gerbils, the antigonadal action of short day (SD; 8L:16D) on reproductive tract weight (RTW; 81.4±8.0 mg vs. 151.8±16.0 mg for long day [$p<0.05$]) was mimicked by a constant dose of melatonin released from two large (3.1 mg) subcutaneous (sc) melatonin pellets (RTW=68.7±3.9 mg). A small (1.0 X 0.4 mm) pellet unilaterally placed in the AH (releasing 90 ng melatonin/day) caused significant reduction in RTW (82.7±6.7 mg) compared to gerbils with a small sc melatonin pellet (129.2±19.7 mg; $p<0.05$) or with a blank pellet in the AH (134.6±14.5 mg; $p<0.05$). The majority of the regressed ovaries lacked a corpus luteum and had significantly fewer mature follicles ($p<0.05$). These results are evidence for a hypothalamic site of melatonin action in this species.

426.6

3-D Mapping of Gonadotropin-Releasing Hormone (GnRH) in Human Basal Forebrain and Amygdala.

E.G. Stopa, E.T. Koh*, C.N. Svendsen*, W. Rogers, J. Schwaber, and J.C. King, (SPON: L.S. Adelman) Depts., of Path. and Anat. and Cell Bio., Tufts Univ. Sch. of Med., Boston, MA; Dept. of Anesth., Univ. of MA, Sch. of Med., Worcester, MA; E.I. Dupont Corp., Wilmington, DE; McLean Hospital, Belmont, MA.

Immunocytochemistry performed on 80um unembedded tissue sections was used to study the localization of GnRH-containing neurons and fibers in the basal forebrain and amygdala of 2 adult female human brains. Sections were subjected to computer-assisted image analysis to generate a three-dimensional map of immunoreactive structures. Cell bodies were concentrated in the preoptic area and basal hypothalamus, but were also evident in the supra-optic nuclei, septal region, anterior olfactory area, and cortical and medial amygdaloid nuclei. GnRH-containing fibers were observed within the hypothalamus (predominantly infundibular region and lamina terminalis), septum, stria terminalis and stria medularis, ventral globus pallidus, dorso-medial thalamus and medial and lateral olfactory stria. Many fibers could also be seen coursing along the base of the brain between the hypothalamus and cortical and medial amygdaloid nuclei. The localization of GnRH-containing cells and fibers in several of these areas represents new observations in the human brain, and suggests an important role for the amygdala in the regulation of gonadotropin secretion in man. (NIA:1K11AG00295)

426.7

MECHANISM OF ACUTE LH SECRETORY RESPONSE TO GNRH. J.-C. Hwan and M.E. Freeman*. Dept. Bio. Sci. Fla. St. U. Tallahassee 32306

GnRH stimulates LH release from gonadotrophs by initially binding to its receptor on the plasma membrane followed by activation of calcium channels and release of LH by exocytosis. In dispersed pituitary cells, studies of LH release in response to long term exposure to GnRH (>4h) have suggested the presence of a readily releasable pool and mechanisms for **de novo** protein synthesis. In the present study, we describe the mechanism involved in short term (<30 min) release of LH following challenge with GnRH. Within 30 min of exposure, the enzymatically dispersed rat pituitary cells release LH in two pulses. The first appeared at about 7 min while the second appeared at 17 min. This pattern was not apparent in the absence of GnRH. Although intracellular LH remained constant within the first 30 min, the ratio of LH secreted into the media versus total LH retained the biphasic pulsatile pattern. Preincubation with colchicine (7.5 uM), which inhibits the polymerization and cross-linking of microtubules, prevented both episodes of LH release within 30 min. Inhibition of protein synthesis by cycloheximide (1 mM,30 min) did not interfere with either phase of short term LH release. Release of LH by depolarization with KCl(59 mM) started only after about 17 min of exposure. Cheleating of calcium ion by EDTA(4 mM) did not interfere with either pulse of GnRH-stimulated LH release. In summary, dispersed pituitary cells responded to the challenge of GnRH with 2 pulses of LH release within 30 min. Thereafter, LH release increased linearly up to 4 hours. Though the cytoskeleton is involved in short term release, calcium ions are not required.

426.8

MECHANISM OF ACTION OF GONADOTROPIN-RELEASING HORMONE (GnRH) AS REVEALED BY STUDIES OF INTRACELLULAR CALCIUM IN SINGLE IDENTIFIED GONADOTROPES. Gary A. Shangold*, Shawn N. Murphy*, and Richard J. Miller (SPON: Morton E. Goldberg). Depts. of Pharm./Phys. and Ob./Gyn., Univ. of Chicago, Chicago, IL 60637.

Regulation of gonadotropin secretion by GnRH has been shown to involve calcium ion (Ca^{++}) influx through voltage-sensitive calcium channels (VSCCs). To elucidate the mechanisms whereby Ca^{++} participates in this stimulus-secretion coupling, we studied $[Ca^{++}]_i$ signals in single gonadotropes from 35-day old female rats, plated on etched-grid coverslips, identified via a reverse hemolytic plaque assay for luteinizing hormone (LH), and loaded with the Ca^{++}-sensitive fluorescent indicator FURA-2, with a microspectrofluorimeter.

Perifusion of cells with GnRH (100 pM) elicited an initial brisk rise in $[Ca^{++}]_i$, which then returned toward basal levels (~100-150 nM), only to rise again to a lower and more sustained secondary plateau phase. At higher concentrations (100 nM), the two phases of the response coalesced to produce a spike / plateau complex. Rapid-time analysis revealed that many cells demonstrated dramatic rapid oscillations in $[Ca^{++}]_i$. The first peak of $[Ca^{++}]_i$ was independent of extracellular Ca^{++}, and thus appeared to reflect mobilization of intracellular stores. The secondary plateau could be prevented by either removal of extracellular Ca^{++} or via VSCC blockade with nitrendipine (Ntd, 10 μM), and was enhanced in a Ntd-sensitive fashion by phorbol esters (TPA, 1μM). Multiple responses to GnRH in a single cell were dependent on intervening rises in $[Ca^{++}]_i$ via influx of extracellular Ca^{++} through VSCCs, which could be brought about either by reintroducing extracellular Ca^{++} into the perifusate, or by cell depolarization with 50 mM K^+. Thus, the secondary (plateau) phase appeared to be necessary for refilling intracellular Ca^{++} stores depleted during the initial (spike) phase.

These data are consistent with a mechanism by which GnRH induces an initial rise in $[Ca^{++}]_i$ via IP_3-mediated mobilization of intracellular Ca^{++} stores, followed by a sustained $[Ca^{++}]_i$ rise due to diacylglycerol / protein kinase C-mediated activation of VSCCs.

426.9

KINETICS OF CRF RNA LEVEL INCREASES AFTER ADRENALECTOMY. R.M. Uht and J. F. McKelvy. Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook N.Y. 11794.

To further characterize the role of glucocorticoids in the regulation of CRF biosynthesis, the increase in CRF RNA observed following adrenalectomy (ADX) and its attenuation by corticosterone (CORT) is being measured over time. Previously, CRF RNA has been shown to increase in the rat hypothalamus after 7 days of ADX (Jingami et al., Endocrinology 117:1314,1985), specifically in the paraventricular nucleus (Young et al., Neurosci. Letts 70:198, 1986; Kovacs et al, Neuroendo. 46:365, 1987). Here, hypothalamic CRF RNA level changes are being evaluated by Northern analyses at 1, 3, 5 and 7 days after ADX. Four groups of rats are being studied: normal, sham ADX + placebo, ADX + placebo, and ADX + corticosterone (CORT), 25 mg, s.c. Serum CORT levels, body weight changes and thymus weights are being measured. To quantitate the CRF RNA changes, total hypothalamic RNA from individual rats is hybridized to a rat CRF probe generated by a random primed reaction using a rat CRF cDNA clone (gift of Robert C. Thompson). In order to correct for the amount of RNA added per lane, Northern blots are stripped and reprobed with a v-ras probe (clone gift of Thomas Roberts). In all treatment groups, at time points evaluated so far (1,3 & 5 days), CRF RNA elutes as a single band at approximately 1.4 kb. CRF RNA is increased by 3 days of adrenalectomy, perhaps as early as by 1 day, and this increase is attenuated by the administration of CORT pellets. That an increase is observed as early as 3 days and is attenuated by CORT indicates that the effect of glucocorticoid removal is more rapid than previously described.

426.10

DIFFERENTIAL REGULATION OF ANTERIOR AND INTERMEDIATE PITUITARY LOBE POMC mRNA OCCURS BOTH AT THE TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL LEVEL. D. J. Autelitano*, M. Lopingco*, M. Blum and J. L. Roberts. Fishberg Research Center in Neurobiology, Mt. Sinai Med. Ctr., New York, NY 10029.

Although chronic treatment with CRF raises the level of anterior lobe (AL) POMC mRNA, several studies have shown an opposite effect in neurointermediate lobe (NIL). In an effort to elucidate the molecular mechanisms involved in this differential regulation we have examined changes in POMC gene transcription, levels of primary transcript and mature mRNA following in vivo treatment with CRF.

Adult female Sprague-Dawley rats (~200g) were injected s/c with 20μg r-CRF or vehicle and sacrificed after 30', 60' and 4hr for acute studies. For long term studies, rats were similarly injected, twice daily for 7 days. An antisense RNA probe spanning all of POMC exon I and part of intron A was used in solution hybridization / S1 nuclease protection assays to quantitate levels of primary transcript, nuclear mRNA and cytoplasmic mRNA. An in vitro nuclear run-on assay was used to determine POMC gene transcription rate.

In AL, POMC gene transcription rapidly increased to 130% of control after 30', and to 240% after 60' of CRF treatment. Consistent with these transcriptional changes, the level of POMC primary transcript rose rapidly, and was maintained at 200% of control from 60' to 4hrs after treatment. Associated with this increase in primary transcript was a consistent 30-40% decrease in the nuclear pool of mature POMC mRNA in AL. No changes in AL cytoplasmic POMC mRNA were observed following acute treatment with CRF. In contrast to the AL, transcription in NIL was maximally induced to 300% of control by 30', and had decreased to only 150% of control levels 60' after CRF injection. NIL POMC primary transcript was significantly elevated to 150% of control values at 30', but had returned to control levels by 60' post CRF injection.

Following 7d treatment with CRF, 2.5 - 3.0 fold increases in POMC cytoplasmic mRNA, nuclear mRNA and primary transcript were observed in AL. Although levels of POMC primary transcript and nuclear mRNA in NIL were not altered, long term CRF induced a 40 - 50% decrease in cytoplasmic levels of POMC mRNA in this tissue.

The differential effects on POMC mRNA in AL and NIL following long term CRF administration appear to be due initially to a difference in transcriptional responsiveness of the two cell types to CRF. Despite an acute stimulation of POMC transcription and primary transcript levels in NIL, repeated injection of CRF leads to a decrease in cytoplasmic mRNA in melanotrophs, which may in part be due to alterations in processing of primary transcript, and/or increased degradation of mature mRNA.

426.11

BETA-ENDORPHIN MEDIATES CLONIDINE STIMULATED GH RELEASE. T.O. Bruhn, P.A. Tresco*, G.P. Mueller and I.M.D. Jackson. Div. of Endocrinol., Brown Univ./R.I. Hospital, Providence, RI 02903

Clonidine (CLON), an α-2 adrenergic agonist, has been shown to stimulate GH secretion by releasing endogenous GRF, but the precise mechanism has been unclear. Since B-endorphin stimulates GH secretion by increasing GRF release, we explored its role in CLON stimulated GH secretion. B-endorphin antiserum (B-end-AS) as well as normal rabbit serum (NRS) and ACTH antiserum (ACTH-AS) as controls and somatostatin-AS (SRIF-AS) were infused slowly (1 ml) 2 hours before the administration of CLON (100 ug/kg BW:IV) via right atrial catheters. In addition, Naloxone (NAL; 2.5 mg/kg BW:IV) was given 15 minutes prior to CLON in some experiments. Blood samples were taken at 15 min intervals prior to and following CLON administration. CLON caused plasma GH to rise 14-fold to peak levels of 170 ± 36 ng/ml ($p<0.01$) at 30 min. Pretreatment of animals with B-end-AS significantly reduced CLON stimulated GH secretion to 87 ± 30 ng/ml ($p<.05$) at 30 min while ACTH-AS did not change GH secretion (197 ± 57 ng/ml). NAL pretreatment significantly blunted CLON stimulated GH release paralleling B-end-AS data (76 ± 20 ng/ml). Pretreatment with SRIF-AS significantly elevated basal GH secretion without changing CLON's ability to increase plasma GH. Conclusions: These results suggest that α-2 adrenergic activation stimulates GRF secretion, at least in part, via B-endorphin as a mediator.

426.12

ASSOCIATION OF SRIF BINDING SITES WITH GRF CONTAINING NEURONS IN THE RAT ARCUATE NUCLEUS. A. Beaudet, E. Moyse*, G. Tannenbaum, C. Kordon, J. Epelbaum. Department of Neurology and Neurosurgery, McGill University, Montreal, Canada; U.159 INSERM, Paris, France.

Growth hormone releasing hormone (GRF) and somatostatin (SRIF) are two neurohormones which directly control growth hormone secretion. In an attempt to clarify the relationship between SRIF receptors and GRF neurons in the brain, we have compared the radioautographic distribution of specifically labeled SRIF binding sites with the immunohistochemical distribution of GRF-containing neurons in sections of rat hypothalamus. SRIF binding sites were labeled in vitro using ^{125}I-Tyr0-DTrp8-SRIF (^{125}I-SRIF). GRF-immunoreactive neurons were visualized in a second group of rats by the PAP method. In radioautographs of sections incubated with ^{125}I-SRIF, the label was concentrated over small, round or oval neuronal perikarya clustered within the ventrolateral aspect of the arcuate nucleus. The distribution of these ^{125}I-SRIF-labeled cells was virtually identical to that of GRF-immunoreactive neurons detected in the same region. Moreover, the number of ^{125}I-SRIF-labeled cells was highly correlated with that of GRF-immunoreactive ones throughout the rostro-caudal extent of the nucleus (r=0.84). These results suggest that SRIF binding sites may be directly associated with the perikarya of arcuate GRF neurons. Such an association would provide an anatomical substrate for the physiological interactions postulated between SRIF and GRF in the hypothalamus. Supported by the MRC.

427.1

POSTNATAL CHANGES IN THE ELECTRICAL PROPERTIES OF MUSCLE-IDENTIFIED RAT MOTONEURONES: AN *IN VITRO* STUDY.

R. Navarrete*, K. D. Walton and R. Llinas. Dept. Physio. & Biophysics., New York Univ. School of Medicine, 550 First Ave., NY, NY 10016.

The functional properties of motoneurons are known to match those of the muscle fibres they innervate with remarkable precision. In order to assess the inductive role of motoneuron activity in muscle fibre differentiation, the membrane properties of identified motoneurons were studied in the *in vitro* neonatal (P0-12) rat hindlimb-spinal cord preparation. Motoneurons innervating soleus, extensor digitorum longus (EDL) and tibialis anterior (TA) muscles were tested. Action potentials recorded from young pups (P0-4) were clearly distinguishable from those of older pups (P6-12) by their marked afterdepolarization (ADP) and prolonged afterhyperpolarization (AHP), reflecting the presence of prominent Ca and K-Ca condutances. The ADP often reached spike threshold eliciting doublet firing. Compared to cells at P6-10, those at P2-4 had a higher input resistance (25 $\pm$ 3.4MΩ, n=9; 15$\pm$ 2.5MΩ, n=16), longer time constant (2.7 $\pm$ 0.3ms n=5; 1.8 $\pm$ 0.1ms n=11), and longer AHP (186 $\pm$ 22ms, n=6; 85 $\pm$ 17ms, n=13). Both in presumptive slow (soleus) and fast (EDL/TA) motoneurons a clear decrease in AHP duration was apparent with age and at this time it appears that the two populations may be separated using this parameter. The changes in membrane electrical properties are probably due to changes in channel density and distribution as well as morphological factors. The functional significance of such changes with respect to muscle differentiation and development of firing patterns will be discussed.

Supported by NIH grant NS-22975 and the Hershel Trust.

427.2

INTRACELLULAR RECORDINGS FROM MOTONEURONS DURING FICTIVE LOCOMOTION IN THE RAT SPINAL CORD IN VITRO.

B.J.Schmidt, J.C.Smith and J.L.Feldman. Dept of Medicine, Univ of Manitoba, Winnipeg Canada R3E 0W3, and Systems Neurobiology Lab, Dept of Kinesiology, UCLA, Los Angeles CA 90024.

Previous work has demonstrated that the *in vitro* neonatal rat brainstem-spinal cord is capable of generating rhythmic ventral root activity similar to motor output patterns recorded during locomotion in preparations with intact limbs (Smith et al, The FASEB J 2:2283-88,1988). The *in vitro* mammalian spinal cord offers the potential to explore neurochemical events underlying spinal locomotor systems which would not otherwise be feasible *in vivo*. In order to further investigate the validity of this preparation as a model of mammalian locomotion, intracellular records were obtained from lumbar motoneurons during fictive locomotion induced by the bath application of N-methyl-DL-aspartic acid or by electrical stimulation of dorsal root afferents. Rhythmic membrane potential oscillations that were phasically related to ventral root burst activity were regularly observed. Some neurons displayed bursts of firing superimposed on the depolarized phase of these drive potentials. These findings are similar to those reported in mammals during locomotion *in vivo*. The effect of intracellular chloride injection, membrane hyperpolarization and bath application of strychnine on inhibitory conductances associated with the oscillating potentials is currently being investigated. The *in vitro* spinal cord of the neonatal rat appears to be a useful model for the study of the spinal networks underlying the generation of locomotion in mammals.

427.3

DISCHARGE PATTERNS OF MEDULLARY RESPIRATORY NEURONS IN MAMMALIAN BRAINSTEM *IN VITRO*

J.C. Smith and J.L. Feldman. Systems Neurobiology Laboratory, Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

In vitro preparations of neonatal rat brainstem-spinal cord generate respiratory motor patterns of brainstem origin (*J. Neurosci. Meth. 21*: 321, 1987). Our preliminary brainstem transection and electrophysiological mapping experiments in this preparation indicate that respiratory neuron populations distributed in the ventral half of the medulla in regions near and ventral to the nucleus ambiguus, retrofacial and facial nucleus are sufficient for motor pattern and rhythm generation *in vitro*. The temporal discharge patterns of respiratory neurons in these regions were characterized by extracellular and intracellular recording. Extracellular recordings were obtained with dye filled microelectrodes (7-10 MΩ); intracellular recordings were obtained with ultra-fine tip (100-140 MΩ) microelectrodes. Populations of inspiratory (I) and expiratory (E) neurons with the following discharge patterns were identified: (i) I neurons with spike discharge onset coincident with or immediately preceding I phase spinal motor output; (ii) neurons with spike discharge that brackets the I phase but are silent during the I phase; (iii) E neurons with early E phase or with continuous E phase discharge. Intracellular recordings show 5-10 mV rhythmic membrane potential oscillations underlying spike discharge in I neurons with temporal characteristics that in part account for the neuronal discharge patterns. Studies in progress are attempting to establish roles of the identified neuron populations in respiratory rhythmogenesis and premotor burst pattern generation.

Supported by NIH Grant NS 24742.

427.4

CHARACTERISTICS OF MOTOR PATTERNS FOR LOCOMOTION GENERATED BY NEONATAL RAT SPINAL CORD IN VITRO.

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Rhythmic motor patterns for locomotion can be generated by chemical or sensory activation of spinal networks in neonatal rat spinal cord in vitro (Smith et al. FASEB J. 2:2283,1988). In order to more precisely characterize the locomotor patterns in vitro, the activation patterns of select flexor and extensor hindlimb muscles were determined from electromyographic recordings. Fine wire electrodes were inserted in the tibialis anterior (TA), lateral gastrocnemius (LG), vastus lateralis (VL), and rectus femoris (RF) of one hindlimb in spinal cord preparations retaining innervated hindlimbs in vitro. The spinal cord circuitry was activated by dopamine, N-methyl-D-aspartic acid, or L-aspartic acid in the presence of the excitatory amino acid uptake inhibitor dihydrokainic acid. Chemically-induced activation patterns were compared to those generated by sensory stimulation (tail-pinch). Comparisons were also made between in vitro locomotor patterns and those during treadmill locomotion in adult rats. The motor patterns in vitro consisted of multi-joint limb movements in which flexor and extensor muscles were reciprocally activated. The temporal relationships among the TA, LG, VL and RF were similar to those recorded during treadmill locomotion in the adult rat, although the cycle periods were much longer and there were variations in the chemically-induced patterns. The excitatory amino acids tended to enhance flexor muscle activity, whereas, extensor muscle activity was enhanced with dopamine suggesting a differential role of excitatory amino acid and dopaminergic mechanisms in locomotor pattern generation. These data establish that spinal motor pattern generating circuitry can be selectively activated in vitro to produce locomotor patterns resembling those generated in vivo.

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427.5

CURRENTS MODULATING EXCITABILITY OF PONTINE RETICULAR NEURONS.

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There is evidence that neurons of the medial pontine reticular formation (mPRF) mediate the essential events of REM sleep. On passage to REM sleep these neurons depolarize 7-10 mV and exhibit a dramatic increase in firing rate. Thus, it is of interest to examine voltage sensitive currents modulating excitability and affected by this state dependent depolarization. Neurons from mPRF slices of young rats were recorded in the presence of TTX, employing a single electrode voltage clamp. We have described two physiological types of mPRF neurons: nonburst (NBN) and low threshold burst (LTBN). In NBNs (n=3) a transient outward current sensitive to 4-AP (500 uM) was observed with activation and inactivation voltage sensitivities similar to those described for I_A. In LTBNs (n=7) an inward current, absent in 0 mM Ca, 10 mM Mg, with voltage sensitivities similar to T current was observed (K currents blocked with Ba, 4-AP, TEA). Similar voltage sensitivities suggest an interaction between these two opposing currents in LTBNs, a point supported by the effects of 4-AP. These currents are kinetically separable because the time required for removal of inactivation of the inward current is greater than that for the transient outward current. Thus, when the membrane potential is depolarized, short duration hyperpolarizations may be followed by a rebound relative refractory period and long duration hyperpolarizations by a rebound low threshold burst.

427.6

CONVERGENCE OF INPUTS FROM THE HINDLIMB AND NECK ONTO NEURONS IN THE LUMBAR SPINAL CORD.

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We recorded extracellularly, in decerebrate cats, from spontaneously active L4 neurons whose activity was modulated by neck rotation, and studied the effects of stimulation of ipsilateral hindlimb nerves. Most units received convergent excitatory or inhibitory inputs from several nerves. The effective muscle nerves were quadriceps (37/43 units), sartorius (19/21) and tibialis anterior (17/34); stimulation of biceps posterior-semitendinosus, biceps anterior-semimembranosus, or gastrocnemius rarely influenced the firing of the neurons. Inputs from quadriceps and sartorius to about half of the neurons were short in latency (< 5ms from the arrival of the group I volley in L5) and from group I or group II afferents, with group II effects far more frequent (cf. Edgley and Jankowska, J. Physiol. 385, 1987). Muscle inputs to other neurons were long in latency and usually had thresholds in the group III range. Cutaneous and mixed nerves were very effective; the central latency was usually > 5 ms.

All of the neurons studied were located ventrally, predominantly ventromedially, in the gray matter. The locations of neurons receiving short-latency group I or II muscle inputs were not segregated from those of neurons receiving higher threshold and longer latency inputs.

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427.7

RECURRENT INHIBITION OF INTERNEURONS MEDIATING VESTIBULAR EXCITATION OF LUMBAR SPINAL RENSHAW CELLS. H.-G. Ross. Department of Physiology, University Düsseldorf, F.R.G.

Dynamic natural stimulation of the maculae modulates the discharge pattern of lumbar spinal Renshaw cells (Ross, H-G & Wittrock, C, Pflügers Arch. 408; R54, 1987). The present study pursues the question how ongoing activity of motoneurons can be integrated into this vestibulospinal pathway. In precollicularly decerebrate cats (no relaxation, dorsal roots intact), motor activity was simulated by electrical shocks to cut ventral roots L7 or S1, while the discharges of individual Renshaw cells (recorded with implanted micro-electrodes) were monitored during transient (falling) or cyclic (quasi sinusoidal) vertical movements of the animals (amplitude 35-40 cm, peak velocity 70-100 cm/s). Of the two phases of modulation (facilitation and inhibition of Renshaw cells) occurring during such natural macular stimulation, only the facilitation was affected by antidromic conditioning excitation of alpha motor axons, being reduced to about 50% of its test value. As judged by its duration (up to 500 ms), this effect cannot be due to antidromic inhibition of motoneurons or to a post-excitatory depression of the Renshaw cells. Hence, it is concluded that Renshaw cells, in addition to their known connections, also form part of an inhibitory feedback loop to those interneurons through which they receive excitation from the vestibular maculae. Supported by the Deutsche Forschungsgemeinschaft; SFB 200

427.8

MULTIPLICITY OF VESTIBULOSPINAL PATHWAYS TO THE UPPER CERVICAL SPINAL CORD OF THE CAT. A.H. Donevan*, M. Neuber*, and P.K. Rose, Department of Physiology, Queen's University, Kingston, Ontario, K7L 3N6.

Descriptions of the anatomical organization of the pathways linking the vestibular nuclei and the motoneurons supplying neck muscles are either incomplete or inconsistent. In the present study, we have taken advantage of the ability of the anterograde tracer, PHA-L to label axons and their collaterals in Golgi-like fashion in order to re-examine the vestibulospinal (VS) connections to the upper cervical spinal cord of the cat.

In each experiment, PHA-L was injected into a discrete region of the vestibular nuclei. These injection sites included regions of the medial, lateral and descending vestibular nuclei. In contrast to previous studies, VS axons were not restricted to the ventromedial and ventrolateral funiculi but were also stained bilaterally in the lateral funiculi, the dorsolateral funiculi and in the dorsal columns. Boutons were also found bilaterally and were located in lamina II through lamina IX. Although the projections of VS axons stained following injections in different sites overlapped, no two injections resulted in identical projections. This suggests that there may be a topographical link between the location of the injection and the distribution of axons and boutons. Regardless, VS projections are more widespread than previously recognized (supported by MRC of Canada).

427.9

THE SUPERIOR COLLICULUS AND THE TRIGEMINO-NECK REFLEXES OF THE CAT, V.C. Abrahams, E.D. Downey*, A.A. Kori*. Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

One of the most basic of head movements in the cat is reflex withdrawal. Consistent with this, a substantial discharge of neck motoneurons follows stimulation of the trigeminal nerve, the trigemino-neck reflex (TNR). The superior colliculus (SC) receives extensive connections from the trigeminal system and has long been presumed to play a role in the organisation of head aversion movements. The reported latency of the TNR is sufficient for the reflex pathway to go through the SC. The TNR has now been more extensively analysed and evidence for an SC pathway examined. In ketamine/chloralose anaesthetised cats the exposed superior colliculus was cooled while recording the TNR. No statistically significant effects either on latency, duration, peak amplitude or the form of the TNR were seen. Ablation of the superior colliculus too was without statistically significant effect on the TNR. The SC may play a role in controlling the excitability of the TNR. Microstimulation of the superior colliculus at current strengths of 20 μA was found to influence the amplitude and latency of the TNR.

Supported by MRC of Canada.

427.10

THE LATERODORSAL AND PEDUNCULOPONTINE TEGMENTAL NUCLEI (LDT & PPT) SEND CHOLINERGIC PROJECTIONS TO THE PONTINE GIGANTOCELLULAR TEGMENTAL FIELD (PFTG) IN THE CAT. A.Mitani, K.Ito, Y.Mitani, A.E.Hallanger, B.H.Wainer, K.Kataoka and R.W.McCarley. Lab. Neuroscience, Dept. Psychiatry, Harvard Medical School/Brockton VAMC, Brockton, MA 02401.

Microinjection of cholinergic drugs into the pontine reticular formation of the cat activates a state with all of the components of natural REM sleep but the sites of origin of any natural cholinergic input have remained unknown. To determine these we used choline acetyltransferase (ChAT) immunohistochemistry combined with retrograde transport of horseradish peroxidase conjugated with wheat germ agglutinin (WGA-HRP) from small (10-30 nl) pressure injections in pontine PTG in 4 cats (TMB processing, 2 days survival). ChAT immunoreactivity was detected utilizing monoclonal antibody AB8 and the PAP method, with DAB as the chromogen. Cell counts showed that 10.2% of the total population of ChAT-labeled LDT neurons were double-labeled ipsilateral to the WGA-HRP injection site in PFTG and 3.7% contralateral. Of all ChAT-immunoreactive neurons in the PPT, 5.2% were double-labeled ipsilateral and 1.3% contralateral to the WGA-HRP injection site. An anterograde study employing a single iontophoretic injection of PHA-L (visualized by the PAP method after 5-8 days survival, 10 cats) showed that PHA-L-positive fibers with bouton-like varicosities were present in both ipsilateral and contralateral PFTG for both LDT and PPT injections, with greater density for LDT.

427.11

HOMOSYNAPTIC DEPRESSION IS SUBSTANTIAL IN SOLEUS BUT INFREQUENT IN VASTUS MEDIALIS. **J.D. Brooke, A.J. McComas[+], P. Yoon, M.K. Wilson and W.E. McIlroy Human Biology/Biophysics, University of Guelph and Department of Neuroscience[+], McMaster University Hamilton, Ontario Canada.**

Homosynaptic depression (HD) of soleus, from pairs of stimuli spaced 50 ms and 300 ms apart, was powerful in both young and old men (n=21). It was anticipated that a weaker heteronymous Ib reflex, exciting tonically contracted vastus medialis (VM) oligosynaptically, would be even more depressed by the repetitive stimulation. However, no such VM depression occurred. Evaluation of homonymous H reflexes in VM revealed HD insensitivity. To test whether pre-synaptic inhibition differentially evoked the soleus HD, weak and strong stimuli were presented separately and together. The proportionality of HD to size of first pulse mitigated against this pre-synaptic hypothesis. The factor differentiating the two muscles is not clear. Tonic contraction is not responsible, as in soleus it did not stop the HD. The differentiation of the muscles may be in after hyperpolarization and neurotransmitter turnover states. **Supported by The ALS Society (Canada) and NSERC grant # A0025.**

428.1

RECEPTOR IMPULSE INTERVAL PATTERNS DEFINE EQUIVALENT OLFACTORY STIMULI. R. C. Gesteland, Anatomy & Cell Biology, Univ. of Cincinnati Med. Ctr., OH 45267.

When an appropriate stimulus is presented to the frog olfactory epithelium those cells which respond do so with a brief increase in action potential (spike) rate. At low stimulus concentrations this results in a few evoked spikes followed by normal spontaneous activity. As concentration increases, the number of evoked spikes increases as does their frequency, resulting in a shorter and more vigorous response. This is followed by a period of depressed excitability which increases in duration with concentration. With some stimulus chemicals at high concentrations as few as 1 or 2 spikes are evoked before the onset of the quiet period. These phenomena are not due to adaptation in receptor-transducer processes Rather, they result from axon inactivation due to ion concentration changes in the olfactory nerve which are not rapidly reversed. There are two useful consequences of these observations. 1. Stimulus equivalents can be established for different stimuli. Equivalent stimuli are those concentrations of various substances which evoked equal spike activity and no subsequent period of suppression in an unadapted nose. 2. At higher concentrations, where suppression follows excitation, durations of suppression periods are a measure of the extent to which the stimulus evokes activity in neighboring axons. At one extreme, the stimulus affects only one cell in a local region, suppression is brief and due only to ionic changes following activity in and around that cell. At the other extreme, the stimulus activates a large proportion of the population, suppression is long lasting and is a measure of the number of cells stimulated and their sensitivities. Such measurements may allow description of cell selectivity and of population coding of olfactory information.

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428.2

ACTIVITY PATTERNS IN THE RAT OLFACTORY SYSTEM REVEALED BY 3-D RECONSTRUCTION OF 2-DG AUTORADIOGRAPHS. G.A. Bell[1], A.H. Reisner[2*], H. Panhuber[1*], K.M. Easton[1*], D. Rosenfeld[3*] and K.K. Tsui[3*]. CSIRO, Divisions of [1]Food Processing and [2]Biotechnology, N. Ryde, Australia, 2113, and [3]School of Engineering, University of Sydney, Sydney, Australia, 2006.

The [14C]2-deoxyglucose method was used to produce autoradiographs from the olfactory epithelium, and forebrain of rats stimulated with clean or cage air, or known concentrations of the odorants ethyl acetoacetate, propionic acid or amyl acetate. The WIP package (Tsui and Rosenfeld, Proc.SPIE,767:654, 1987) was used to digitize images into a 256^2 array (256 grey levels). After background subtraction and calibration against autoradiographic standards, the images were aligned in a 256^3 array, using DIANA software (Hibbard et al., Science,236:1641, 1987) and utilities provided by the Fleurs Control Program package (Sch.Elec.Eng., U.Syd.). Three dimensional activity maps were deduced for various radiation thresholds using software developed for the VAX/VMS from the DISPLAY 82 package written by G.T. Herman et al. for analysis of CT scans (Proc.SPIE, 367:3, 1982). At low activity thresholds the epithelial maps closely resemble the complex morphology of the rat nasal turbinate region. At higher levels, the relationship between active zones and nasal geometry can be observed.

428.3

PROPERTIES OF THE OLFACTORY GENERATOR CURRENT. S. Firestein, F.S. Werblin and G.M. Shepherd, Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510

Recordings of the generator current in olfactory sensory neurons were obtained with the whole cell patch clamp technique. The use of isolated cells (no mucus), and a technique which permitted us to monitor the magnitude and time course of a pressure ejected stimulus solution at the ciliary membrane, have allowed us to make several fundamental measurements of the odor generated current. 1) Dose-Response Relations. Odorous substance concentrations required for threshold responses were in the 10^{-5} Molar range. Dose-response curves followed a signmoidal shape and were very steep, saturating over less than a log unit of stimulus concentrations ($K_{\frac{1}{2}} = 2\times10^{-4}$ M). 2) Response latency. The mean latency from the arrival of the odorous substance to the onset of the current was 350 msec, range = 150-650 msec. The mean latency from the peak of the stimulus concentration to the peak of the current response was 650 msec. 3) Desensitization. The response to a maintained stimulus (20 sec) was transient with a decay half time of 9.4 seconds. Responses to short (100 msec) but saturating stimulus steps every 5 seconds showed only a 20% decrement after 20 consecutive stimulations. Supported by NS 07609 and NS 10174.

428.4

DEVELOPMENT OF A METHOD FOR PRIMARY RAT OLFACTORY NEURON CULTURE. J. Pevsner(1), G. Ronnett(1,2), L.D. Hester(1) and S.H. Snyder(1), Depts. of Neuroscience(1) and (2)Neurology, Johns Hopkins Medical Institutions, 725 North Wolfe Street, Baltimore, MD 21205.

The olfactory neuron has been thus far refractory to primary cell culture. We describe a method which yields a nearly pure olfactory neuronal population. Turbinates from 2-3 day neonatal rats are removed, transferred to MEM, and finely minced. The tissue is pelleted at low speed and incubated for one hr at 37°C in MEM containing digestion enzymes. Thereafter, the tissue is pushed through a wine screen, and pelleted. Cells are resuspended in MEM, pelleted, and resuspended in MEM-D-valine containing 15%/° dialyzed fetal calf serum and 10 μM Ara C. Cell clumps are removed by filtering through 50 μM Swinex filters. The resulting single-cell suspension is plated on laminin-coated dishes. After 5 days, 98%/° of cells remaining are bipolar, one process being long and unbranched while the other is short and divides into 6 to 8 branches.

These cells stain for olfactory marker protein (OMP) and vimentin, but are negative for keratin, GFAP, S-100, and neurofilament. Based on these and other markers, these cells appear to be neuronal with no evidence for glial, fibroblast and epithelial cellular contaminants. These cultures to our knowledge represent the first instance of olfactory neurons maintained in pure culture.

428.5

SINGLE-CHANNEL K^+ CURRENTS IN THE APICAL MEMBRANE OF NECTURUS TASTE CELLS. S.C. Kinnamon. Department of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523 and Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center,4200 E. 9th Ave., Denver, CO 80262.

Previous studies have shown that isolated mudpuppy taste receptor cells possess a voltage-dependent K^+ current which is modulated by sour taste stimuli (Kinnamon and Roper, J. Gen. Physiol. 91:351-371, 1988) and is restricted to the apical membrane (Kinnamon et al., Biophys. J. 53:11a, 1988). In this study, single-channel recordings in the cell-attached and inside-out configurations were used to identify the types and voltage-dependence of the K^+ selective channels involved in this conductance. A large number of K^+ channels was found on the apical membrane (classified by conductance values of approximately 27, 46, 55, 80, 90, 100, 110, 120, 132 and 180 pS). It is not clear if all of these represent different channels, or if some values represent variability of a single channel type. However, there are at least 5 different conductance channels because that many different channels were observed in a single patch. All channels showed a small probability of opening at the resting potential, and the smaller channels showed an increased probability of opening with depolarization. The largest channel was Ca^{++}-dependent as well as voltage-dependent. Studies are underway to determine the effects of taste stimuli on the different K^+ channels. (Supported by NIH grant NS20382).

428.6

mRNA TRANSLATION OF PUTATIVE ODORANT-GATED ION CHANNELS IN OVO. T.V. Getchell. (SPON: M. Getchell). Dept. Anat. Cell Biol., Wayne St. Univ. Sch. of Med., Detroit, MI 48201.

Total mRNA was isolated from olfactory mucosae (30 μg/130 mg tissue) of Rana pipiens and from 14 d old rat pup brains (8.2 mg/8 brains). About 150 ng olfactory mucosal mRNA/50 nl sterile water, about 50 ng rat brain mRNA/50 nl sterile water or 50 nl sterile water was microinjected into individual mature oocytes isolated from Xenopus laevis. Oocytes from each group were non-responsive to an odorant mixture and other ligands 2 d later. On the 3rd day, oocytes microinjected with Rana pipiens olfactory mucosal mRNA responded to an odorant mixture but not to other ligands; oocytes microinjected with rat brain mRNA responded to 5-hydroxytryptamine but not to an odorant mixture. The controls were non-responsive to the odorant mixture. The magnitude of the small-amplitude (10-20 nA), long-duration (2-3 min) current transients recorded in response to odorant stimulation was enhanced by preincubating the oocytes with 1 mM 8-bromo cyclic AMP for 10 min. The data suggest that odorant-gated ion channels associated with sensory transduction in olfactory receptor neurons were translated and inserted into the oocyte membrane. Further experiments will determine the usefulness of the Xenopus oocyte as a convenient and efficient translation system for mRNAs encoding membrane receptors, intermediate molecular entities and channel proteins associated with olfactory transduction. Experiments performed at Cold Spring Harbor Laboratory; supported by NIH-NS-16340.

428.7

CYTOCHEMICAL LOCALIZATION OF Na^+, K^+-ATPase IN GERBIL OLFACTORY EPITHELIUM. R.C. Kern*[1], T.P. Kerr*[1], and T.V. Getchell[2]. Depts. of Otolaryngology[1] and Anatomy & Cell Biology[2], Wayne State University, Detroit, MI 48201.

Olfactory epithelium consists of bipolar sensory neurons, sustentacular and basal cells. The apical knobs of the neurons, with appended cilia, are the presumed sites of odorant binding. Receptor potentials measured across this epithelium are sustained, at least in part, by active Na^+ transport. The identity and distribution of the sodium transport mechanism(s) therefore have significance for transduction. Since the enzyme Na^+, K^+-ATPase mediates active sodium flux in a variety of other systems, we have used a cytochemical technique (Ernst, J. Histochem. Cytochem., 1972) to identify sites of elevated Na^+, K^+-ATPase activity in olfactory epithelium. The procedure detects phosphate, which is released from an artificial substrate by enzyme catalytic activity, then converted to a product visible in the electron microscope. In tissues incubated for demonstration of Na^+, K^+-ATPase activity, reaction product was associated preferentially with the apical knobs, cilia, and dendrites of receptor neurons. In controls incubated with the specific Na^+, K^+-ATPase inhibitor ouabain, or with substrate deleted, only a small quantity of non-specific precipitate was observed. Our demonstration of elevated enzymatic activity in the apical portion of the receptor neuron extends recent biochemical data (Anholt, et al., J. Neurosci., 1986). Sodium flux in this region of the neuron, associated with odorant binding, may necessitate a high level of enzymatic activity to maintain the cation gradients required for sensory transduction. [Supported by NIH-T32-NS-07305, NIH-NS-16340 (TVG), and a Wayne State University Research Grant (TPK)].

428.8

PERIRECEPTOR EVENTS IN THE OLFACTORY ORGAN OF THE LOBSTER: EFFECTS ON THE DETECTION OF GLUTAMATE, TAURINE, AND PURINE NUCLEOTIDES. H.G. Trapido-Rosenthal, R.A. Gleeson, K.A. Cottrell, J.T. Littleton, S.L. Wachocki, and W.E.S. Carr. The Whitney Laboratory, U. of Florida, St. Augustine, FL 32086

The olfactory organ of the Florida spiny lobster, Panulirus argus, consists of an array of aesthetasc sensilla on the lateral branch of the antennule. Each sensillum is innervated by several hundred chemosensory neurons. Electrophysiological studies show that among these neurons are cells that respond specifically to glutamate, taurine, AMP, or ATP. It is presumed that each cell type has receptors on the cell surface that interact with the molecules that induce electrophysiological responses.

Biochemical studies demonstrate that perireceptor events occur in the sensilla which may affect the ability of odorants to interact with receptors. These events include specific, Na-dependent uptake systems that internalize the amino acid odorants, thus removing them from the receptor environment. The activity of the uptake system for glutamate appears to be enhanced by the presence of taurine. The excitatory nucleotides AMP and ATP are dephosphorylated by specific ectonucleotidases that require the presence of divalent cations. The sensillar 5'-ectonucleotidase converts AMP to the completely inactive nucleoside Ado, whereas the product of the ATPase, ADP, is a compound that strongly inhibits ATP-sensitive cells. We propose that these perireceptor events may play an important role in the olfactory process of this marine invertebrate.

428.9

CHEMOSPECIFIC DEFICITS IN TASTE DETECTION FOLLOWING SELECTIVE GUSTATORY DEAFFERENTATION. A. C. Spector*, G. Schwartz* and H. J. Grill (SPON: C. R. Gallistel). Dept. of Psychology and Monell Chem. Senses Ctr., Univ. of Penn., Phila., PA 19104.

Historically, most attempts to measure changes in the rat's gustatory sensibility following selective gustatory deafferentation have involved the exclusive use of intake tests. These tests are contaminated by non-taste factors. Moreover, intake tests have questionable sensitivity in the peri-threshold concentration range. It is therefore not surprising that researchers have found only modest or no deficits in intake preference exhibited toward NaCl and sucrose following rather extensive gustatory deafferentation. Using a conditioned avoidance procedure in which small volumes of taste stimuli serve as a signal for shock, we have found a significant 2 log unit increase in the NaCl threshold, but only a marginal increase in sucrose threshold following deafferentation of only 15% of the taste buds by bilaterally sectioning the chorda tympani nerve. The effect of bilateral glossopharyngeal nerve section (removes 64% of taste buds) on sucrose and NaCl thresholds is under investigation. The psychophysical data collected in these experiments, along with other behavioral data from our laboratory (Nitabach, this session) and recent electrophysiological findings from others, are consistent with the suggested existence of Na+ specific receptors that are primarily limited to the receptive field of the anterior tongue of the rat.

428.10

GUSTATORY IDENTIFICATION OF SODIUM IONS IS DEPENDENT ON THE CHORDA TYMPANI INNERVATION OF THE RAT TONGUE. M. Nitabach*, G. Schwartz* and H. Grill. Dept. of Psychology, University of Pennsylvania, Philadelphia, PA 19104.

The geniculate ganglion contains cells that are uniquely responsive to sodium ions. Behavioral data from this lab (Spector, this session) suggest that of the 2 neural inputs to this ganglion, the chorda tympani provides the exclusive input channel for the gustatory identification of sodium. The present study uses a paradigm developed by Nachman (1962) to further test this hypothesis. 24 male Sprague-Dawley rats were given a 10 min. 2-bottle preference test. The solutions presented were 0.4M NaCl and 0.2M NH_4Cl; cumulative salt preference (cum. 0.2M NaCl intake/total cum. fluid intake) was measured after each minute of the test period. At the time of testing all rats were 24 hr. water deprived. Rats were divided into 2 groups, intact (n=12) and bilateral chorda tympani sectioned (CTX, n=12). Within each of these 2 groups, intact and CTX, half of the rats were sodium replete and half were sodium deplete at the time of the preference test. For the intact group, salt preference was clearly a function of internal state. Sodium replete rats preferred the NH_4Cl solution; sodium deplete rats preferred the NaCl solution. Within each condition (replete or deplete), the preference must have been based solely on the orosensory properties of the two salt solutions; all preferences became significant within the first minute of the test, too soon for post-ingestive effects to have occurred. Results for the CTX group differed markedly; internal state did not alter salt preference. Both the sodium replete and sodium deplete rats preferred the NH_4Cl solution. This failure of the sodium deplete CTX rats to prefer the NaCl solution can best be explained by a deficit in the gustatory identification of sodium.

428.11

GENETIC FACTORS IN TASTE BUD DENSITY AND TASTE PREFERENCE. I.J. Miller, Jr. and G. Whitney*. Dept. Anat., Wake Forest Univ., Winston-Salem, NC 27103 and Dept. Psychol., Florida State Univ., Tallahassee, FL 32306-1051.

We hypothesize that some differences in taste sensitivity may be attributable to variation in taste bud density. The SWR/J strain of mice avoids the bitter substance sucrose octaacetate (SOA) at concentrations above .01 mmolar, and the C56BL/6J strain is indifferent to SOA at the same concentration. Electrophysiology yields smaller responses to SOA in the glossopharyngeal nerves of non-taster mice compared with tasters. We have undertaken a comparison of taste bud density in vallate papillae of these two strains of mice. Serial, paraffin sections were made and complete taste bud counts were compared. The SWR/J (taster) strain contained a mean of 169.4 (N=5, range 142-217) taste buds/ vallate papilla,and the C57BL/6J (non-taster) strain averaged 140.1 (N=10,range 102-179) taste buds/ vallate papilla. These distributions are probably significantly different ($t=-1.843$, $df=13$, $p<.05$). While the factors which determine taste preference in mice are probably more complicated than the density of taste buds in one population, a greater number of taste buds in tasters may produce a more intense taste response near the threshold of taste perception. (Supported by NIH Grants NS 20101 and NS 15560).

428.12

THE CAROTID BODY CHEMORECEPTORS AS POLYMODAL SENSORS. J. Alcayaga*, R. Iturriaga* and P. Zapata. Lab. of Neurobiology, Catholic University of Chile, Santiago 1, CHILE.

All the sensory fibers of the carotid (sinus) nerve innervating the carotid body parenchyma respond to chemical changes of the blood (pO_2, pCO_2, pH), leading to their naming as chemosensory fibers. However, these afferents are also responsive to thermal and osmotic stimuli. We studied their possible responses to changes in flow and their interactions with oxygenation and temperature. Cat's carotid bodies were superfused with modified Tyrode's solution, buffered with HEPES-NaOH to pH 7.41 at 37.7°C. The frequency of discharges from the entire carotid nerve was determined at steady flow rates between 0.15 and 2.95 ml/min. The best fit for the regression of basal chemosensory activity on superfusion flow was provided by inverse sigmoid curves, resulting in maximal gains at about 0.78 and 0.86 ml/min, at 100% and 20% O_2 levels, respectively. The peak frequency evoked by 5 min flow interruptions was maximal and independent of previous superfusion flows, but the half-excitation time of responses to flow interruption in 100% O_2 equilibrated saline was minimal when preceded by superfusion at 0.7 ml/min. The response to flow interruption was also evoked under 100% N_2 equilibrated solutions, suggesting that a mechanism different from hypoxia participates in its generation. The changes in basal chemosensory activity in response to thermal increases in 1°C steps between 36 and 39°C were dependent on superfusion flows. It is concluded that the carotid nerve chemosensory discharge frequency may be determined by flow, when all other natural chemoreceptor stimuli are held constant. Thus, the carotid body chemoreceptors may act as detectors of steady levels and transient changes of low and temperature. Supported by DIUC, FONDECYT, and Gildemeister Foundation.

429.1

PROTEIN KINASE C ACTIVITY DURING CEREBRAL ISCHEMIA IN RAT. R.C. Crumrine*, J.C. LaManna, G. Dubyak*.(SPON: W.D. Lust). Depts. of Physiology/Biophysics and Neurology, Case Western Reserve University Medical School, Cleveland, OH 44106

Protein kinase C (PKC) can be activated by increased intracellular Ca2+, increased availability of diacylglycerol, and free arachidonic acid. All of these changes in the intracellular environment have been described in cerebral ischemia. In this study, we investigated the consequences of cerebral ischemia on activation of PKC. Cerebral cortex samples were obtained from rats at control and after 5, 10, 15, 20, and 30 minutes of irreversible cerebral ischemia produced by KCl induced cardiac arrest. Rats were frozen in situ at the times indicated. We did not observe an increase in PKC activity in the membrane over the time course studied, suggesting that PKC is not activated during ischemia. However, there was a significant increase in activator-independent activity in the membrane fraction after 5 minutes of ischemia. There was also a rising trend in the proportion of activator-independent activity in the membrane. PKC can be converted to an activator independent form known as protein kinase M by proteolytic cleavage secondary to attack by calpains. We propose that this may be occuring during ischemia. We also noted a general trend of decreasing inducable total PKC activity over the ischemic time course studied. Activator independent kinase activity did not correspondingly rise. This may reflect a change in PKC efficacy or a disappearance of the enzyme.

429.2

SELECTIVE VULNERABILITY OF NEURONS TO HYPOXIA-ISCHEMIA: ROLE OF CALCIUM-BINDING PROTEIN. G.G. Wasterlain*, W.Massarweh*, A.Sollas*, E.Rouk* and R.S.Sloviter. VA Med. Ctr., Sepulveda, CA 91343, UCLA Sch. of Med., and Coll. Phys. Surg., Columbia Univ. and H.Hayes Hospital.

In 7-day-old rats, bilateral carotid ligation and exposure to 8% O2 for 60 min causes extensive neocortical and hippocampal damage. The inner layer of hippocampal granule cells (HGC) is selectively vulnerable, while CA3 pyramids are resistant. HGC vulnerability decreases with age and after 18 days of age they are resistant to the same treatment, while CA3 becomes vulnerable. Immunocytochemical stains with polyclonal antibodies to calbindin D28K (calcium-binding protein, CABP) show that the vulnerable inner layer HGC at 7 days and the vulnerable CA3 cells of the adult lack CABP, while the resistant outer HGC and CA3 cells at 7 days contain CABP. While this association does not prove causality, it is compatible with a role of CABP as a buffer that limits rises in intracellular free calcium and protects neurons during hypoxia-ischemia. Supported by grants NS13515 and NS18201 from NIH and by the research service of the VA.

429.3

A NOVEL CALCIUM CHANNEL BLOCKER AND SEROTONIN S2 ANTAGONIST, (S)-EMOPAMIL, MARKEDLY REDUCES INFARCT SIZE IN RATS WITH MIDDLE CEREBRAL ARTERY OCCLUSION. H. Nakayama*, M.D. Ginsberg, W.D. Dietrich. Cereb. Vasc. Dis. Research Ctr., Univ. of Miami Sch. of Med., Miami, FL 33101

Reports of neuroprotection conferred by calcium channel (CC) blockers in cerebral ischemia have been variable and inconsistent. In this study we assessed (S)-emopamil (E), a highly permeable agent with both CC and serotonin S2 blocking properties. In 32 fed adult male Sprague-Dawley rats, focal cerebral ischemia was induced by electrocoagulating the proximal middle cerebral artery (MCA). In 3 groups, (S)-emopamil, 10 mg/kg in distilled water, was injected IP either 1) 30 min before, 2) immediately after, or 3) 1 hr after MCA occlusion. The same E dose was given twice a day until perfusion-fixation at 3 days. Brain infarct areas were measured by planimetry and volumes computed by integration.

INFARCT VOLUME (mm^3, mean±S.D., n=8 per group)

	Control	Pre-Rx	Post-Rx 0hr	Post-Rx 1hr
CORTEX	86±22	33±23	29±14	27±16
STRIATUM	30± 6	25±11	36± 4	27± 3

In the cortex, E reduced infarct volume to 31-38% of control ($p<0.01$) and pre- and post-ischemic regimens were equally effective. The effect was most apparent at the infarct periphery. Striatal infarct volume was not altered by E. (S)-emopamil appears to have great promise in treating focal cerebral ischemia.

429.4

RECEPTOR MEDIATED MODULATION OF HYPOXIA-INDUCED CALCIUM ACCUMULATION IN CULTURED NEURONS. A.W. Probert, M.L. Weber, and F.W. Marcoux. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

NMDA receptor mediated excitotoxicity has an important role in hypoxic neuronal injury in vitro. It has been proposed that neuronal calcium accumulation is pivotal in this process. We examined the effects of NMDA antagonists and other receptor modulators on hypoxia-induced Ca^{++} accumulation in cultured neurons. Cortical isolates of fetal rat brain were exposed to a 95% N_2/5% CO_2 atmosphere for four hours. Just prior to hypoxic exposure, maintenance medium was replaced with Hanks' balanced salt solution containing varying concentrations of test compounds. Intracellular calcium accumulation was quantitated by adding trace amounts of $^{45}Ca^{++}$ to the test medium. TTX (3 µM)/Mg++ (10 mM) controls were used to estimate hypoxic Ca^{++} accumulation in glial cells.

The competitive NMDA antagonists 7-APH and CPP blocked $^{45}Ca^{++}$ accumulation with IC50s of 10 and 4µM, respectively. The non-competitive NMDA antagonists, ketamine and MK-801 inhibited influx with IC50s of 2 and 0.03 µM, respectively. Each antagonist suppressed calcium accumulation to near TTX/Mg++ control levels. NMDA antagonists prevent hypoxia-induced Ca^{++} accumulation in cultured neurons.

429.5

MONOSIALOGANGLIOSIDE GM1 REDUCES ANOXIC NEURONAL DEGENERATION IN VITRO. S.D. Skaper, L. Facci, D. Milani* and A. Leon. Fidia Research Laboratories, Abano Terme, Italy.

Glutamate neurotoxicity (GNT) may participate in the neuronal cell losses associated with neurological insults such epilepsy, anoxia and stroke. GNT has been described in cortical and hippocampal neurons in vitro, and cell death under hypoxic conditions. In cultured cerebellar granule cells, GNT has been reported to be prevented by pretreatment with gangliosides GT1b, GD1a, and GM1 (Favaron, M. et al., FASEB J., 2:A824, 1988). We now report ganglioside effects in anoxic-exposed cultures of granule cells from day 8 rat cerebellum. Chemical anoxia, in cells between 8 and 12 days in vitro, was produced by a pulse addition of rotenone (0.1 µM for 1 hr) in the absence of Mg^{2+}. Widespread degeneration of neuronal cell bodies and their neurite network was seen the following day. This neuronal injury was abolished by the specific NMDA receptor antagonist PCP, suggesting an action of endogenous glutamate at this receptor. Pre- or concurrent treatment (>30 min) with ganglioside GM1 or its inner ester derivative largely prevented (>70%) the ensuing neuronal death, even after 4 days (ED50= 25 µM); degeneration by added glutamate was likewise reduced. These results support the observed beneficial effects of the gangliosides in ischemic brain injury in vivo.

429.6

MK-801 PROTECTS THE HIPPOCAMPAL SLICE FROM HYPOXIC INJURY IN THE ABSENCE OF EXTRACELLULAR CALCIUM. J.E. Parsons,* M.D. Fairchild,* R.A. Wallis* and C.G. Wasterlain* (SPON: N. Rosenthal). Epilepsy Res. Lab., VAMC, Sepulveda, CA 91343, Dpt. Neurology and Brain Res. Institute, UCLA Sch. of Med.

MK-801, a potent noncompetitive NMDA receptor antagonist, reduces neuronal hypoxic injury. We tested the role of extracellular calcium in this process, using hippocampal slices perfused with artificial cerebrospinal fluid (ACSF) containing (mM) CaCl2, 2.4 or 0; glucose, 4; and saturated with 95% O2/5% CO2 (normoxic) or 95% N2/5% CO2 (hypoxic). One hour after 40 min of hypoxia in 2.4 mM Ca, slices recovered 12%± 8 of the orthodromic population spike (OPS) evoked in CA1 by Schaeffer collateral stimulation. MK-801 (10 mg/l) in 2.4 mM Ca during hypoxia increased recovery to 100%. Normoxic slices in 0 Ca for 210 m. recovered 70%± 11 of OPS. 40 min hypoxia in 0 Ca yielded a recovery of 22%± 10 OPS in the absence, and 91%± 6 in the presence of MK-801 (10 mg/l). The protective effect of MK-801 in the absence of Ca in the perfusate is difficult to reconcile with its most commonly accepted mechanism of action through receptor-operated channels. Alternative hypotheses could include kinase C-mediated release of intracellular Ca stores, influx of other ions through receptor-operated channels, or modulation of metabolic rate.

429.7

HYPERGLYCEMIA PROTECTS CULTURED ASTROCYTES FROM HYPOXIA. G.A. Gregory*, A.C.H. Yu*, and P.H. Chan. Departments of Anesthesia and Neurology, University of California, San Francisco, CA 94143.

Hyperglycemia increases central nervous system (CNS) damage in animals and man, possibly by increasing lactic acidosis and decreasing pH or by increasing brain osmolarity. To determine which of these factors cause damage, we studied the effects of glucose concentration [Glc] and osmolarity on primary cultures of astrocytes that were obtained from the cerebral cortex of newborn rats. Four to six week old cultures were exposed to 0, 1.5, 3.0, 4.5, 6.0, 7.5, 15, 30, or 60 mM Glc and 95% N_2/5% CO_2 for 24 hrs at 37°C and 100% humidity. Other cultures had 7.5 mM Glc and 0, 4.5, 7.5, 15, and 30 mM mannitol added and were treated similarly to the Glc-treated cultures. After 24 hrs of hypoxia, the culture medium (MEM) was decanted, and the cells were washed with MEM and dissolved in 1 M NaOH. The concentration of lactate dehydrogenase (LDH) in the MEM from hypoxic cells was determined as an index of cell injury. Cell damage was also assessed morphologically by phase-contrast microscopy. All of the Glc disappeared in 24 hrs from the medium of cultures that initially contained 1.5, 3.0, 4.5, and 6.0 mM Glc. About 80% of the Glc disappeared when 7.5 mM Glc was added and about 2/3 disappeared when 15, 30, and 60 mM Glc was added to the MEM. Lactate progressively increased to a maximum of 16.7 ± 3.5 mmol/L (60 mM Glc added). The pH of the MEM after 24 hrs of hypoxia was 6.92 ± 0.04. The concentration of LDH in the MEM was 110-270 mmol/L in cultures containing 0-7.5 mM Glc, while that of cultures containing 15, 30, and 60 mM Glc was not different from normoxic controls (29 ± 1 mmol/L). The LDH of mannitol-treated cultures was not different from those treated with Glc alone. Hypoxic cells treated with 0-7.5 mM Glc or with Glc (7.5 mM) + mannitol appeared swollen, vacuolated, or completely disrupted, while those treated with 15-60 mM Glc appeared normal by phase-contrast microscopy. Our data suggest that neither hyperglycemia nor hyperosmolarity increased the amount of hypoxia-induced cell damage of primary cultures of astrocytes. Supported by NS-14543, NS-25372, and NS-26092.

429.8

FOCAL BRAIN ISCHEMIA REVERSES THE NORMAL INTERSTITIAL/INTRACELLULAR pH RATIO M. Nedergaard*, R.P. Kraig, J. Tanabe*, and W.A. Pulsinelli (SPON: F. PLUM). Department of Neurology Cornell University Medical College NY, NY 10021.

Intracellular pH (pH_i) is tightly regulated in all eukaryotic cells and normally is more acid compared to interstitial pH (pH_e). The purpose of this study was to examine whether focal ischemia influenced the relationship between pH_e and pH_i. Microelectrodes were used to measure pH_e and (^{14}C-5,5-dimethyl-2,4-oxazolidione (DMO)) was used to measure tissue pH in rats with focal stroke. This allowed calculation of regional pH_i where pH_e was measured. Focal cerebral ischemia was produced in 6 spontaneously hypertensive rats by occlusions of the right common carotid and middle cerebral arteries under halothane anesthesia; 15 min later, 50 uCi ^{14}C-DMO was injected and allowed to circulate for 1 hr. In the left, non-ischemic cortex the pH_e/pH_i ratio was 1.04 (7.24±0.02/ 6.98± 0.02; mean±SEM). In the area of dense cortical ischemia, the pH_e/pH_i ratio of 0.94 (6.44± 0.07/ 6.82± 0.08) was reversed and significantly different ($p<0.01$, student-t test) from normal. The one hour of focal ischemia in these brains caused histologically detectable infarction in the core ischemic zone. Results from this study suggest that 1) some population of cells in the evolving infarct remains sufficiently intact to maintain a pH_e/pH_i gradient albeit reversed and 2) cortical average pH_i may be little altered from normal in tissue undergoing infarction.

429.9

REDUCTION OF STROKE SIZE BY ELECTRICAL STIMULATION OF THE FASTIGIAL NUCLEUS OF THE CEREBELLUM IN RAT. *M. Khayata*, S. Berger, M.D. Underwood and D.J. Reis (SPON: J. Grebb).* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Electrical stimulation of the cerebellar fastigial nucleus (FN) elicits a global increase in regional cerebral blood flow which, in the cerebral cortex, is not associated with any change in metabolic rate (Nakai, et al., Brain Res., 260:35-49, 1983). We sought to determine whether FN stimulation produces a protective effect on cerebral ischemia. Focal cerebral ischemia was produced by unilateral occlusion of the middle cerebral artery (MCA) in forane anesthetized Sprague-Dawley rats (0.5-1% in 100% O2). The MCA was occluded by electrocautery medial to the inferior cerebral vein. Arterial pressure (AP) and blood gases were continuously monitored and maintained within a normal range. Twenty four h later the animals were sacrificed, their brains removed and frozen in freon, and sections collected through the entire lesion and stained for nissl. In unstimulated controls, MCA occlusion produced an infarction with a mean ischemic area of 241 ± 37 mm^2 (n=9). Sham operated animals had a smaller area of infarction 78 ± 9 mm^2 ($p < 0.01$; n=6). To determine whether the infarct area was reduced by FN stimulation, we electrically stimulated the FN (50 Hz; 1 sec on/1 sec off; 50-100 μA) for 1 h. FN stimulation increased AP and heart rate (threshold for 10 mmHg increase in AP = 31 ± 6 μA; maximum increase: 27 ± 2 mmHg at 100 μA). Following MCA occlusion and FN stimulation, AP was maintained within autoregulated range for CBF by blood withdrawal. FN stimulation reduced the mean ischemic area to 148.74 ± 23.42 mm^2 ($p < 0.05$; n=7). These findings suggest that FN stimulation protects against focal cerebral ischemia and results in a 38% reduction in the size of the infarct produced by MCA occlusion. This reduction in infarct size with FN stimulation may represent a rescue of the ischemic penumbra by a drop in vascular resistance and opening of collateral flow.

429.10

IMPROVING CEREBRAL BLOOD FLOW DOES NOT IMPROVE METABOLIC STATE IN PERINATAL ASPHYXIA. W.B. Stewart, L.R. Ment*, O.A.C.Petroff*, & C.C.Duncan*. (SPON: W.F. Collins, Jr) Depts. of Pediatr., Neurol., Surg., & Anat., Yale Univ. School of Medicine, New Haven, CT 06510.

During perinatal asphyxia cerebral blood flow (CBF) is markedly reduced in the gray & white matter of the telencephalon. We tested the hypothesis that a thromboxane synthesis inhibitor (TSI) would improve CBF & blunt metabolic alterations that accompany asphxyxia. Newborn beagle pups (2-7 d) were anesthetized, ventilated & randomized to insult (I = 5 min of asphyxia) or no insult (NI) & treatment with the TSI CGS 13080 (Ciba Geigy Corp) (0.06 mg/kg/hr i.v. infusion) or saline (S). Pups received TSI/S 30 min prior to I/NI. In S/I pups, CBF increased during insult in the medulla (100.9±10.7 ml/100 g/min, mean ± S.D.) but decreased elsewhere (cortex 25.0± 4.0, white 11.7±2.7). TSI/I pups increased CBF during insult in all regions (medulla 271.2±28.2, cortex 60.0± 4.0, white 25.0±7.8). In other pups, brain extracts were prepared for 1-H NMR spectral analysis of high energy phosphorylated compounds & lactate levels. In S/I pups, PCr fell from 1.6±0.2 to 0.1±0.1 mmole/kg, lactate increased from 1.2±0.5 to 4.0± 0.8 & pH fell 0.9 units. There were no differences between S/I & TSI/I. These data suggest therapeutic strategies for perinatal asphyxia must extend beyond CBF maintenance to methods which may reduce metabolic needs & prevent pathologic change.

429.11

70 KDa STRESS/HEAT SHOCK PROTEIN INDUCTION IN GERBIL BRAIN AFTER ISCHEMIA IS BLOCKED BY THE ANTICONVULSANT, MK-801. T.S. Nowak, Jr. Laboratory of Neuropathol. and Neuroanat. Sciences, NINCDS, NIH, Bethesda, MD 20892

Immunocytochemical studies have localized induction of the 70 KDa stress protein (hsp70) in gerbil brain after transient ischemia. Accumulation is most striking in CA3 neurons of hippocampus at 48 h recirculation, preceding the loss of CA1 neurons characteristic of this model. MK-801 has been suggested to block ion channels associated with glutamate receptors of the N-methyl-D-aspartate subtype and thereby protect neurons from excitotoxic cell loss. The present study examined the effect of MK-801 on hsp70 induction and CA1 damage following bilateral carotid artery occlusion in the gerbil. Pretreatment with 10 mg/kg MK-801 1 hr before 5 min ischemia prevented the appearance of hsp70 immunoreactivity in CA3 at 2 d, and posttreatment at 1 hr notably attenuated the induction. In contrast, no protection of CA1 neurons was evident after either treatment when evaluated histologically at 1 week. These results fail to support a protective effect of MK-801 on postischemic cell damage in CA1, and clearly dissociate the induction of hsp70 in CA3 from the pathophysiology of hippocampal cell loss. On the other hand these observations demonstrate that hsp70 induction is subject to pharmacological intervention, and provide a basis for further studies of the physiology resulting in such localized changes in gene expression after ischemia.

429.12

OPIOID PEPTIDES IN FOCAL CEREBRAL ISCHEMIA IN THE CAT. D.S. Baskin* and J.L. Browning (Spon: DMK Lam). Neurosurgery Department and the Center for Biotechnology, Baylor College of Medicine, Houston, TX 77025.

Opioid peptides(OP) have been implicated in the pathophysiology of stroke. Baskin etal (Nature) found that naloxone and OP can improve neurologic function and prolong survival. We measured OP concentrations following induction of focal cerebral ischemia using intracranial vascular occlusion in cats. Met enkephalin(ME), leu enkephalin(LE), dynorphin 1-17(DYN) and beta-endorphin (BE) were measured at six hours post occlusion. OP were separated using RP- HPLC were assayed using RIA. ME was increased in caudate (CAU) and cortex (CTX) and decreased in hypothalamus(HYP). LE likewise increased in CAU but also increased in hippocampus (HIP), amygdala, and thalamus with dimuition in the HYP. No significant increases were seen in DYN concentrations in any area, with decrease seen in CTX. BE levels increased in HIP, thalamus and HYP and decreased in frontal CTX. These data document changes in OP following ischemic injury, including increases in regions with a loss of cellular function. As the motor depressive effects of OP are well described, changes described here may partially explain the attenuation in motor function seen in stroke, as well as the change in motor function observed after administration of OP antagonists. This is supported by other data demonstrating that OP antagonists afford no protection from ischemic injury, despite improvement in motor function.

430.1

A NOVEL METHOD FOR TARGETING NEURONS IN A LIGHTLY FIXED STRIATAL SLICE PREPARATION. R.H. Walker*, A.M. Graybiel, R.W. Baughman and G.W. Arbuthnott*. (SPON: H. Newman-Gage) Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139; Dept. of Neurobiol., Harvard Medical School, Boston, MA 02115.

Intracellular filling of cells in the in vitro tissue slice provides detailed information about their dendritic fields. Methods have been developed for targeting specific cells by prelabeling them with transportable retrograde dyes or with nonspecific nuclear dyes such as 4,6-diamidino-2-phenylindole (DAPI). We here report a method for using a fluorescent Nissl stain (see Quinn and Weber, this meeting) that permits targeting of specific cell types according to the appearance of their perikarya, combined with injection of Lucifer Yellow, visible at the same wavelength. This method was developed in a lightly fixed striatal slice preparation. Slices 400μm thick were taken from perfused ferret brain and maintained in a bath on the stage of a microscope fitted with a 470nm epifluorescence filter. Meta-phenylene diamine was applied topically to produce a fluorescent Nissl stain. Cells with different perikaryal dimensions were targeted with a micropipette and Lucifer Yellow was injected under direct vision into the cell of choice. After a number of cells had been filled in a slice, it was post-fixed by immersion in 4% paraformaldehyde-phosphate buffer, and cut into 100μm thick sections which were then stained for cholinesterase. This method enables reconstruction of dendritic fields of striatal cells with respect to the histochemically definable striosomes, and should have broad applicability to the study of other regions of the central nervous system. *Supported by the Faculty of Medicine, Univ. of Edinburgh, the Whitaker Health Sciences Fund and NSF BNS 8720475.*

430.2

AN AFTERHYPERPOLARIZATION IN NEOSTRIATAL CELLS RECORDED IN VITRO: EFFECT OF DOPAMINE. M. Garcia-Munoz, A. Rutherford, and G.W. Arbuthnott, MRC Brain Metabolism Unit, University of Edinburgh, United Kingdom.

A slow afterhyperpolarization (AHP) following a train of action potentials has been described in mammalian central neurons. Is there an AHP in striatal cells? If so, what is the effect of dopamine (DA) on the AHP? Parahorizontal 300 μM slices of rat neostriatum were maintained at the fluid interface of a perfusion chamber at 36°C. Cells were recorded intracellularly (potassium acetate, 60-90 Mohms). No spontaneous activity was observed. The mean resting membrane potential was -83.7 ± 6.4 mV (n=82), depolarizing pulses (0.5-1 nA/80 ms) produced action potentials which overshot 0 mV. After a train of action potentials an AHP followed in cells depolarized from a holding potential of -65 to -70 mV. The input membrane resistance did not change during the course of the AHP. The estimated reversal potential at 4.5 mM external potasium was -90± 4 mV. Iontophoresis of DA (0.5 M; 25-50 nA/3-5 min) resulted in several distinct actions: 1) As already reported (Calabresi et al., 1987), it reduced the membrane resistance with depolarization increasing the quantity of injected current necessary to reach the voltage of the firing level. 2) Induced accommodation to repeated cell firing. 3) Decreased the size of the AHP with an associated increase in the number of spikes. These results may help to reconcile the excitatory versus inhibitory effects of DA previously reported in the literature.

430.3

SHIFTS IN CLASS DISTRIBUTION OF TH-LABELED CELLS IN THE SUBSTANTIA NIGRA OF THE NEUROLOGICAL MUTANT MOUSE WEAVER. D.E. Smith, M.W. Smith, III*, T.R. Cooper*, and T. H. Joh. Depts. of Anatomy & Biometry, LSU Med. Ctr., New Orleans, LA 70112 and Dept. of Neurol., Cornell Univ. Med. College, New York, NY.

The neurological mutant mouse weaver is best known for its cerebellar deficit. However, there is now evidence that tyrosine hydroxylase (TH) labeled neurons within the substantia nigra are also affected. The present study was undertaken to determine if there are specific subpopulations of TH-labeled neurons within the substantia nigra that are more severely depleted in the weaver than in the homozygous wildtype control.

Weaver and control mice were perfused with fixative. Thirty micron vibratome sections were immunocytochemically labeled with a rabbit antiserum to TH. Chi-square analyses comparing weaver with wildtype controls indicate that the number of immunocytochemically-labeled cells is significantly lower in weaver than in controls while the number of labeled cells with areal measurements of 20-74 microns and maximum diameters of 61-69 microns is significantly higher. (Supported by BRSG SO-RR 5376)

430.4

REGIONAL EFFECTS OF THE WEAVER GENE ON PRESYNAPTIC DOPAMINE UPTAKE SITES IN STRIATUM. S. Roffler-Tarlov and A.M. Graybiel, Neuroscience Program, Tufts Univ. Sch. Med., Boston, MA 02111 AND Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

The reduction of endogenous dopamine resulting from effects of the weaver gene in mice is remarkably specific, discriminating between the nigrostriatal and mesolimbic pathways so that the nigrostriatal targets are profoundly affected and the mesolimbic targets are relatively or completely spared. We report here measurements of ^{3}H dopamine uptake, a reflection of dopamine-containing terminal function, in the three striatal regions in weavers and controls. Synaptosomes were prepared from caudoputamen (CP), nucleus accumbens (NAc) and olfactory tubercle (OT). Three animals per sample were pooled for NAc and OT. Aliquots of synaptosomes were incubated with .05 μM ^{3}H dopamine. The specificity of ^{3}H dopamine accumulation was assessed with nomifensin. The results revealed an even greater deficit in uptake capacity than in dopamine content in weaver's CP (16% vs. 31% of control) and in OT (40% vs. 52% of control). In the weaver's NAc, where dopamine content is 108% of control, accumulation of ^{3}H dopamine was reduced to 68% of control.

These results show that deficits in ^{3}H dopamine uptake exceed reduction of endogenous dopamine in the most vulnerable regions of the weaver's striatum, and that terminal function is significantly compromised also in the NAc, a mesolimbic target in which dopamine content is normal. This evidence suggests that effects on terminals may be a key feature of the action of the weaver gene on the dopamine-containing mesostriatal pathways. Supported by NS20181 and MH00655.

430.5

TOWARD A GENETIC ANALYSIS OF THE STRIOSOMAL SYSTEM: PATTERNS OF NIGROSTRIATAL LOSS IN THE MUTANT MOUSE WEAVER. A. M. Graybiel, K. Ohta* and S. Roffler-Tarlov. (SPON: D. Chikaraishi) Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139; Neuroscience Program, Tufts Univ. School of Medicine, Boston, MA 02111.

The weaver (wv) mouse carries an autosomal recessive gene leading to defects in the central nervous system. In the basal ganglia, the dopamine (DA)-containing nigrostriatal system is severely affected. With the glyoxylic acid method we have shown that the loss of DA innervation is greatest in the dorsolateral quadrant of the caudoputamen, with its ventral part least affected. We now report evidence that tyrosine hydroxylase (TH)-immunoreactive fibers are lost not only from the dorsolateral quadrant but also from striosomal regions (identified by low met-enkephalin-like immunoreactivity in serially adjoining sections) within the remaining partially affected parts of the caudoputamen.

Analysis of TH in the wv/wv midbrain suggests highly specific patterns of cell depletion in the A8-A9-A10 complex relative to cellular distributions present in littermate controls: 1) TH-positive neurons are sparse in the midlateral part of the substantia nigra pars compacta (SNpc); 2) there is marked shrinkage of cell group A8; 3) there is nearly total loss of TH-positive neurons in a caudomedially situated hypercellular zone of A9 characterized by its dense immunostaining for TH. We propose that the cellular depletion in the mid-A9 and A8 regions in the wv/wv may account for the massive loss of TH-containing fibers in the caudoputamenal matrix, whereas loss of neurons in the cell-dense caudomedial zone (putative murine densocellular zone) may account for loss in the striosomal system. *Supported by NIH 20181, The Seaver Institute and MH0655.*

430.6

DOPAMINERGIC CONTROL OF STRIATAL GABA SYNTHESIS IS REFLECTED IN THE EXPRESSION OF mRNA FOR STRIATAL GLUTAMIC ACID DECARBOXYLASE. K. Gale, J. Segovia*, N.J.K. Tillakaratne*, K. Whelan* & A.J. Tobin. Dept. of Pharmacology, Georgetown Univ. Med. Ctr., Washington, D.C. 20007 & Dept. of Biology & Brain Research Inst., UCLA, Los Angeles, CA 90024.

Lesions of dopaminergic innervation of the striatum are known to result in a marked increase in striatal GABA synthesis as measured both "in vitro" (glutamic acid decarboxylase, GAD) and "in vivo" (GABA turnover). The present study examined GAD mRNA as related to the increase in striatal GAD activity 3 months after dopaminergic denervation by 6-hydroxydopamine in the MFB. Rats were sacrificed and the striata rapidly removed and divided into 2 pieces, one to measure GAD activity using a radiochemical method, the other to assess GAD mRNA levels. A 32P labeled cloned rat GAD cDNA probe was used to detect striatal mRNA for GAD. Total mRNA was isolated using guanidine thiocynate-cesium chloride followed by electrophoresis in formaldehyde. Northern blot of RNA from striatal tissue revealed a single band at 3.7 kb. Striata from rats that were succesfully denervated showed a marked increase in GAD activity on the lesioned side when compared with the non-lesioned side (between 40 and 100% increase), or with non-lesioned controls. Similar changes could be seen in GAD mRNA levels. We suggest that the increased GAD activity observed after dopaminergic denervation of the striatum is due to a "de novo" synthesis of the enzyme and that striatal dopaminergic neurotransmission has a regulatory function on the GABAergic cells' genome expression.

Supported by NIH grants NS 20576 & NS 20356 and RSDA (K.G.) MH 00497.

430.7

TRANSPLANTS OF FETAL SUBSTANTIA NIGRA CAN REGULATE GABA SYNTHESIS IN THE STRIATA OF ADULT HOST RATS. J. Segovia*, N. Hayman* and K. Gale (SPON: S. Gobel). Dept. Pharmacology, Georgetown Univ. Med. Ctr. Washington, D.C. 20007.

The purpose of the present experiments was to determine whether transplants of fetal nigral tissue are able to reverse the alterations in host striatal GABAergic function produced by 6-hydroxydopamine (6-OHDA). Rats with unilateral 6-OHDA lesions received solid transplants of fetal mesencephalon placed into cortical cavities. 6-8 weeks after transplantation, striata were removed and a radiochemical assay was used to determine glutamic acid decarboxylase (GAD) activity. There was no difference in the GAD activity between the two striata in non-lesioned rats, whereas there was a marked increase (>40%) in striatal GAD activity homolateral to the 6-OHDA lesions in rats without transplants. In contrast, in rats with successful transplants striatal GAD activity was similar to the non-lesioned control values. Successful transplants were defined as those which reduced apomorphine- or amphetamine-induced rotational behavior by at least 50%. Unsuccessful transplants were those without significant effect on this behavior; these were also without significant effect on the lesion-induced increase in striatal GAD activity. This suggests that dopamine released from the fetal tissue graft can reverse the changes in GABAergic activity caused by the 6-OHDA lesion.

Supported by a grant from the United Parkinson Foundation, a PMAF fellowship (J.S.) and an RSDA (K.G.) MH 00497.

430.8

ULTRASTRUCTURAL LOCALIZATION OF MOLECULAR SUBTYPES OF NEURAL CELL ADHESION MOLECULE (NCAM) IN THE ADULT RODENT STRIATUM. M. DiFiglia, P. Marshall* and M. Yamamoto. Departments of Neurology, Massachusetts General Hospital, Boston, MA 02114 and Biochemistry, Eunice Kennedy Shriver Ctr., Waltham, MA 02254.

Neural cell adhesion molecule (NCAM) belongs to a class of integral plasma membrane glycoproteins which are thought to mediate adhesion between neuronal elements. We used immunohistochemistry (avidin-biotin-peroxidase) to examine the localization of immunoreactive (i) NCAM in the adult rodent striatum. The monoclonal antibody used in this study was raised against embryonic (E15-E17) mouse brain and identified 180, 140 kD molecular weight forms of NCAM. Light microscopic results in the adult mouse (N=4) and rat (N=4) showed that iNCAM was localized to the plasma membranes of somata and dendrites of medium and large-sized aspiny neurons throughout the caudate nucleus and to the majority of neurons in the globus pallidus. Ultrastructural analysis revealed that reaction product in aspiny somata was present in discrete, closely spaced patches along the inner face of the plasma membrane and was also prominent in somatic protrusions which were frequently apposed to synapsing axons. Distal aspiny dendrites with iNCAM received numerous synaptic inputs. Within caudate neuropil iNCAM was also present in: 1) medium spiny neurons where reaction product was localized predominantly to spines with, long thin necks and small spine heads which were postsynaptic to unlabeled axon terminals; and 2) preterminal (unmyelinated) axons and terminal boutons that issued from myelinated bundles and formed asymmetric synapses with unlabeled dendritic spines.

We speculate that the prevalence of iNCAM in medium and large aspiny caudate interneurons and in pallidal projecting cells may be involved with modulating the density of synaptic inputs, since all three types of neurons share the feature of being ensheathed by axons and terminals. The presence of iNCAM in thin spines may reveal sites of plasticity in adult spiny neurons. Supported by grants NS16367 to M.D. and NS 24726 to M.Y.

430.9

REGIONAL DISTRIBUTION AND REGULATION OF SOMATOSTATIN MESSENGER RNA (SOM mRNA) IN THE STRIATUM, AS REVEALED BY IN SITU HYBRIDIZATION HISTOCHEMISTRY (ISHH). L.T. Weiss and M.-F. Chesselet. Dept. of Pharmacology, the Medical College of Pennsylvania, Philadelphia, PA 19129.

Using ISHH, we examined the regional distribution of SOM mRNA in the striatum of the mouse. Also, because lesions of dopaminergic nigrostriatal neurons stimulate striatal SOM neurons, we determined the effects of dopamine (DA) receptor blockade on the levels of striatal SOM mRNA. Male Swiss Webster mice were injected 2 times, 8 hours apart, for 2 consecutive days with a dose of fluphenazine-N-mustard (FNM, 4 µmol/kg), which irreversibly blocks DA-D2 but not D1 receptors. The right striata were analyzed for DOPAC and DA using HPLC-EC. The DOPAC to DA ratio, an index of DA turnover, was elevated after FNM treatment, confirming effective blockade of DA-D2 receptors. The left side of the brain was processed for ISHH. Levels of SOM mRNA were quantified using light microscopy and computer-assisted grain analysis. In controls, individual neurons of the lateral striatum had higher levels of SOM mRNA than did those of the medial striatum. FNM treatment produced a decrease in SOM mRNA levels in the lateral, but not the medial striatum. The results indicate that there is a lateral to medial gradient in the levels of SOM mRNA in the striatum of control animals which is abolished by FNM treatment. Taken together with data from the literature, these results suggest that activation of the nigrostriatal pathway by sustained DA-D2 receptor blockade produces a region specific reduction in striatal SOM gene expression. Supported by BNS 86-07645 and BNS 86-16841.

430.10

PERSISTENCE OF POLYSIALYLATED ("EMBRYONIC") N-CAM IN THE SUBSTANTIA NIGRA OF ADULT RATS. M.-F. Chesselet and L.I. Aaron*, Dept of Pharmacology, Med. Coll. Pennsylvania, Philadelphia, PA, 19129.

The embryonic (E) form of the Neural Cell Adhesion Molecule (N-CAM) contains a higher amount of polysialic acid residues than adult N-CAM (A N-CAM). As a result, binding between A N-CAMs is much stronger than between the E forms of the molecule. Maturation from E to A N-CAM is believed to be a critical developmental event, but its chronology has not yet been studied at the anatomical level. We have detected the highly polysialylated N-CAM in sections of the rat brain by indirect fluorescence immunohistochemistry, using a monoclonal antibody (from G. Rougon) raised against the capsular polysaccharides of meningococcus B. This antibody reacts with the E but not the A N-CAM. In the substantia nigra (SN), the labelling was intense and homogenous in sections from 1-10 day old rats, with both cell membranes and intersomata space showing immunoreactivity. At 16 days, a ventro-dorsal gradient in the level of immunostaining appeared in the SN pars reticulata. Also, immunolabelling of cell membranes became increasingly discontinuous, taking on a more punctate appearance in sections of 3 weeks old rats. Immunostaining was still present in the SN of adult rats, but was found mostly between cell bodies. Labelling in the adult SN contrasted with the lack of immunolabelling of most other brain areas at this age. Notable exceptions in the brain stem were the periaqueductal grey and the lateral subnuclei of the interpeduncular nucleus. At all age examined, intense and widespread immunoreactivity to a polyclonal antibody recognizing both A and E N-CAM was found throughout the brain. It is proposed that highly sialylated N-CAM may play a role in neuronal plasticity in discrete areas of the adult brain, including the SN. Supported by BNS 86-07645 and the Dystonia Medical Research Foundation.

430.11

NORADRENERGIC INNERVATION OF THE RAT SUBSTANTIA INNOMINATA: A LIGHT AND ELECTRON MICROSCOPIC STUDY OF DOPAMINE ß-HYDROXYLASE IMMUNOREACTIVE AXONS. H.T. Chang, Department of Anatomy and Neurobiology, The University of Tennessee - Memphis, The Health Science Center, 875 Monroe Ave., Memphis, TN 38163.

Noradrenergic input to the rat substantia innominata (SI) was investigated in this study by immunocytochemical localization of dopamine ß-hydroxylase (DBH), the synthetic enzyme for noradrenaline. Using a rabbit anti-bovine DBH antiserum (Eugene Tech International), DBH immunoreactive (DBH+) elements were revealed by a standard avidin-biotin-HRP immunoreaction. DBH+ axons ramified extensively within SI and appeared to be contiguous with DBH+ terminal fields within the bed nucleus of stria terminalis and the amygdaloid complex. Individual DBH+ boutons in SI appeared much larger than those in the cerebral cortex, hippocampus, and thalamus. Electron microscopic analysis revealed that DBH+ boutons in SI contained many small clear synaptic vesicles (30-60 nm) and large uniformly stained vesicles (80-120 nm). DBH+ boutons formed asymmetrical synapses with mainly dendrites, but also somata and spines of SI neurons. Dendrites which were postsynaptic to DBH+ boutons also formed many symmetrical, and some asymmetrical synapses with unlabeled axon terminals. Since previous studies have shown that dendrites of SI cholinergic neurons formed few synapses, the present result suggests that noradrenergic influence of SI cholinergc neurons, if present, is through polysynaptic connections. (This study was supported by USPHS Grants NS21003, AG05944, and a grant from the Alzheimer's Disease and Related Disorders Association.)

431.1

THE HYPOTHALAMIC GnRH PULSE GENERATOR OF THE RHESUS MONKEY: THE DURATION OF PHASIC ELECTRICAL ACTIVITY AND ITS CONTROL. * C.L. Williams, *M. Nishihara, *J-C. Thalabard, *P.M. Grosser, *J. Hotchkiss, and E. Knobil. Laboratory for Neuroendocrinology, University of Texas Health Science Center, Houston, TX 77225.

The characteristic circhoral increments in multiunit activity (volleys) associated with the initiation of gonadotropic hormone pulses were studied in adult female rhesus monkeys bearing bilateral arrays of chronic recording electrodes placed in the region of the arcuate nucleus (Wilson et al., Neuroendocrinology 39:256, 1984). In the intact animal, during the follicular phase of the menstrual cycle, the duration of the volleys is 1-3 min. During the luteal phase, this is increased to 4-6 min. After ovariectomy, volley duration increases progressively to 15-25 min. Physiological estradiol replacement reverses this effect. In the intact monkey, during the follicular phase, administration of naloxone (0.3 - 1.0 mg/kg i.v.) results in volleys of increasingly longer duration, while morphine (1 - 30 µg/kg i.v.), like estradiol administration to ovariectomized animals, results in a dose-related decrease in volley duration. These findings suggest that the ovarian influence on the duration of the electrical volleys, like that on their frequency as previously described, may be mediated by endogenous opioid peptides.

431.2

THE PREPUBERTAL HIATUS IN PULSATILE GNRH RELEASE IN THE MALE RHESUS MONKEY IS NOT THE RESULT OF OPIOID INHIBITION. R. Medhamurthy*, V.L. Gay* and T.M. Plant. Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

In male primates, pulsatile GnRH secretion is interrupted from shortly after birth until puberty by a mechanism of nongonadal origin. This study investigated the possible role of opioid peptides in determining the prepubertal hiatus in GnRH release in the male monkey. In order to use LH secretion as a bioassay for hypothalamic GnRH release, pituitary sensitivity was heightened in 4 castrated, prepubertal male monkeys (17-18 mo) by an iv intermittent infusion of GnRH. GnRH stimulation was discontinued 10-12h before challenging with naloxone (NAL). In one experiment, NAL was administered as an iv bolus at 4 doses (0, 0.2, 2 and 10 mg/kg BW in saline) and LH was determined in plasma collected at 10-30 min intervals before and after injection of the opioid antagonist. At the low and high NAL doses there was no increase in plasma LH levels. At the 2 mg/kg dose, however, 1 of the 4 animals showed a 2½ fold increase in plasma LH. In another experiment, monkeys were infused either with NAL (2 mg/h) or saline for 36h, and plasma was collected every h. LH levels during NAL and saline infusion were not different. In both experiments, GnRH injection after NAL administration elicited an LH discharge. These results fail to provide convincing evidence that the prepubertal hiatus in pulsatile GnRH release in male monkeys involves opioid inhibition. An additional experiment is now underway to test the effect of long-term intermittent NAL treatment on hypothalamic GnRH release in order to fully substantiate our conclusion.

431.3

PROGESTERONE IS REQUIRED FOR NALOXONE (NAL)-INDUCED LH SURGE. C. Masotto*, A Sahu* and S.P. Kalra, Dept. OB/Gyn, Univ. Fla. Col. Med., Gainesville, FL 32610

Recent studies show that continuous restraint of the hypothalamic inhibitory opioid tone produced by NAL infusion can advance the preovulatory LH surge on proestrus. We have now identified the ovarian steroid signals that facilitate this NAL-induced decrease of opioid restraint. Ovariectomized rats (7-10 days) received estradiol (E_2)-filled capsules (300 g/ml) on day 0. On day 2, saline (SAL, 0.6 ml/h) or NAL (2 mg/0.6 ml SAL/h) was infused between 1100- 1400 h through one and blood samples for LH estimations were withdrawn (between 1000-1700 h) through the other intracardiac cannula. While in SAL-infused rats plasma LH levels were unchanged, surprisingly NAL infusion in these E_2-treated rats induced only a marginal (< 2-fold) increase in plasma LH between 1600-1700 h. However, when E_2-primed rats were additionally treated with progesterone (P, 3 mg, s.c.) at 1800 on day 1, followed by similar NAL infusion on day 2 between 1100-1400 h, a robust LH surge was observed. The onset of the LH surge was advanced by 2 h (LH rise at 1130-1200 h in NAL vs 1330-1400 h in SAL-infused); LH peaks occurred earlier (NAL: 1530-1600 vs SAL: 1630-1700 h) and were of longer duration (NAL: 1530-1700 h vs SAL: 1630-1700 h). Thus, P pretreatment facilitated the neuroendocrine processes involved in induction of the LH surge by NAL in ovariectomized rats. These findings imply that expression of the hypothalamic restraint on inhibitory opioid tone, that normally precedes the proestrous LH surge, requires P action on an estrogen background and that E_2 alone may be an inadequate ovarian signal. (Supported NIH HD 08634).

431.4

PITUITARY LH RELEASE IS REFRACTORY TO NMA STIMULATION IN THE LACTATING RAT. C. R. Pohl, L.-R. Lee*, and M. S. Smith, Dept. of Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Whereas the suppression of LH secretion during lactation has been attributed to inhibition of GnRH release, lactating rats have a normal hypothalamic content of GnRH which is releasable by electrical stimulation. The present study was undertaken to examine whether this system can be activated by chemical stimulation of NMDA receptors, which have recently been shown to function in the control of pulsatile LH secretion in males (Arslan et al., in press).

On day 10 of lactation, OVX rats suckling 8 pups were primed for 24 hrs with GnRH pulses (5 ng iv once every 50 min) to restore pituitary GnRH receptors. Four pulses of NMA (40 mg/kg/pulse) were then infused 50 min apart, and serial blood samples were collected at 10-min intervals. Procedural controls for the NMA infusion regimen (not primed with GnRH) included gonad-intact male rats and estrous females.

LH responses (ng/ml) to 20 mg NMA/kg in males (mean peak LH/mean basal LH: 75/11) were higher than in estrous females treated with NMA at either 20 mg/kg (peak/basal: 34/18) or 40 mg/kg (peak/basal: 58/20). Whereas OVX lactating rats responded to GnRH pulses after 24 hrs of treatment (peak/basal: 51/15), LH responses to subsequent pulses of 40 mg NMA/kg were absent in these animals ("peak"/basal: 16/16). Additional efforts to stimulate LH release with NMA pulses administered over 24 hrs were similarly unsuccessful.

These data indicate a sex difference in LH responsiveness to NMA between the male and the estrous female rat and suggest that the hypothalamus of the lactating rat is refractory to NMDA receptor stimulation.

431.5

PARADOXICAL RATE OF GNRH RELEASE FROM HYPOTHALAMUS OF THE LACTATING RAT. L.-R. Lee*, C.R. Pohl, and M.S. Smith, Dept. of Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

During lactation in the rat, the suppression of pituitary GnRH receptors and pulsatile LH secretion can be reversed by administering pulsatile GnRH. These data suggest that the suckling stimulus suppresses endogenous GnRH secretion. To more directly test this hypothesis, we compared in vitro GnRH release rates from hypothalami of lactating rats suckling 8 pups (day 10 postpartum) and cycling diestrous rats. Individual hypothalami (POA-MBH) or median eminences (ME) were incubated and the medium was exchanged every 10-15 min during 3-5 hours of incubation.

From the ME, basal GnRH release rates (pg/min) were similar in lactation (0.47 ± 0.05) and diestrus (0.34 ± 0.07), and GnRH release rates stimulated by 55 mM K^+ (pg/ml) were about 10-fold higher in both groups: lactation, 4.9 ± 0.5; diestrus, 3.4 ± 0.9. From the POA-MBH, basal GnRH release rates did not differ between lactation (0.40 ± 0.05) and diestrus (0.53 ± 0.19), and GnRH release rates stimulated by K^+ were only 2 to 3-fold higher than basal rates (lactation, 1.3 ± 0.3; diestrus, 1.0 ± 0.4). Addition of verapamil to the medium greatly decreased both basal and stimulated GnRH release, thus the similarity in GnRH release rates between lactation and diestrus cannot be explained by a high rate of nonspecific release. Also, hypothalamic GnRH content was similar in lactating and diestrous rats.

These results are a paradox in view of in vivo studies suggesting that lactation suppresses GnRH release. One explanation is that isolation of the hypothalamus removes it from inhibitory neural input arising from other areas of the CNS activated by the suckling stimulus.

431.6

ELECTRICAL STIMULATION OF THE HYPOTHALAMUS FACILITATES LHRH RELEASE IN THE STALK-MEDIAN EMINENCE (S-ME) OF THE PERIPUBERTAL FEMALE MONKEY AS DETERMINED BY PUSH-PULL PERFUSION. L.E. Claypool, G. Watanabe', and E. Terasawa. Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI 53715

As we have previously reported, the onset of puberty in the female rhesus monkey is heralded by an increase in LHRH release. Preliminary to studies of the mechanism underlying this increase, we have evaluated the LHRH neurosecretory system following electrical stimulation in the peripubertal female (age 25-40 months). LHRH release in the S-ME of conscious monkeys was assessed by push-pull perfusion as described previously. Modified Krebs-Ringer phosphate buffer solution was perfused at a rate of 20 µl/min, and perfusate was collected at 10-min intervals for 12 h. After 3 h of baseline perfusion, electrical stimulation (50 Hz, 0.5 msec, 500 µA) was applied 6 times for 5-10 min at 90-min intervals via a monopolar electrode, the tip of which was placed 1-2 mm rostro-dorsal to the perfusion site. **Results:** Electrical stimulation of the mediobasal hypothalamus (MBH) resulted in 1) a prompt release of LHRH which occurred within 20 min after each stimulation, and 2) a gradual increase in mean LHRH release, possibly due to long-term potentiation. Regarding the latter, mean LHRH release, 2.0±0.7 pg/ml/10 min during the 90 min prior to the first stimulation, ultimately reached 8.2±1.8 pg/ml/10 min during the 90 min after the 6th stimulation (n=8). In contrast, mean LHRH release in controls, 2.1±0.6 pg/ml/10 min prior to sham stimulation, remained stable with ultimate levels of 3.5±1.0 pg/ml/10 min (n=6). These data provide evidence that electrical stimulation of the MBH facilitates LHRH release in the peripubertal monkey by 1) depolarizing LHRH neurons and/or their terminals directly, and 2) stimulating interneurons that synapse on the LHRH neurosecretory system. (Supported by NIH grants HD11355 and HD15433.)

431.7

EFFECTS OF PRAZOSIN ON PULSATILE LUTEINIZING HORMONE (LH) RELEASE IN OVARIECTOMIZED FEMALE RHESUS MONKEYS. M. Gearing and E. Terasawa. Neuroscience Training Prog. and Wisconsin Reg. Primate Res. Ctr., Univ. of Wisc., Madison, WI 53715.

Previously, we have reported that prazosin, an α_1-adrenergic antagonist, suppressed, but did not completely block, pulsatile release of luteinizine hormone releasing hormone (LHRH; Endocrinology 122 *Suppl* #15, 1988). While our results indicating suppression of LHRH by prazosin agree with a previous report of LH suppression from another lab (Endocrinology 116:1327, 1985), the two studies disagree on the nature of the suppression. The following experiments were therefore performed to further examine the effects of prazosin on LH pulses in 8 ovariectomized female monkeys. Serial blood samples were collected from conscious monkeys at 10-min intervals through indwelling veinous catheters. After a 4 h control period, prazosin (0.2 or 0.4 mg/kg) or vehicle was injected i.v., and sampling was continued for 6 h. **Results** Both doses of prazosin induced a biphasic response: an initial period of suppression lasting up to 210 min (0.2 mg/kg) or 250 min (0.4 mg/kg), followed by a "rebound" period which lasted until the end of the experiment. During the suppression period, LH pulses were completely suppressed in only 2 of 8 (0.2 mg/kg) and 1 of 4 (0.4 mg/kg) animals; in the remaining animals, LH pulses continued with attenuated amplitude and normal frequency. During the "rebound" period, LH pulses occurred with higher amplitude and lower frequency than in the control period. No significant dose effects were observed in any aspects of the analysis. Furthermore, injection of the vehicle had no effect on pulsatile LH release. **Conclusion** Prazosin suppresses LH pulse amplitude but not frequency, indicating that an α_1-adrenergic mechanism is involved in pulsatile LH release. This finding is consonant with our previous study of pulsatile LHRH release. (Supported by NIH HD15433 and RR0169.)

431.8

IN VIVO PULSATILE LHRH RELEASE INTO THE ANTERIOR PITUITARY (AP) OF THE MALE RAT. P.S. Kalra, C.P. Phelps[1] and S.P. Kalra, Dept. OB-Gyn, Univ. FL., Gainesville, FL and [1]Dept. Anat., Univ. So. FL, Tampa, FL.

In the intact male rat, LH release is irregular with low amplitude pulses at variable intervals (30 min to >12 h). Post-castration hypersecretion of LH is characterized by an increase in frequency and amplitude driven by the putative LHRH generator. We have studied LHRH release in vivo using push-pull cannulae (PPC) placed in the AP of freely behaving adult male rats. Six rats were perfused (PERF) as intacts (day 0) and again at 1, 2 and 7 d after castration (CAST). Similarly, 12 rats were PERF on day 0 and at 3 and 7 days after CAST. Additional CAST rats were PERF at 14 (n=7) and >40 days (n=6). LHRH levels were analyzed by RIA and subjected to PULSAR analysis. Pulsatile LHRH release (0.83±0.08 pulses/h; amplitude 0.54±0.13 pg) was detected in 16 of 18 intact rats (average release rate 0.53±0.08 pg/10 min) and in 45 of 48 CAST rats; there were no significant differences in the pulse parameters of intact vs CAST rats. Although in rats subjected to repeated PERF before and after CAST there was a progressive increase in the number of rats exhibiting increased pulse amplitude and frequency, the actual levels of these parameters were not significantly different at any interval after CAST. In one group of 5 CAST rats pulse amplitude increased to 1.61±.31 pg ($p < .05$) on day 7. These results demonstrate no change after CAST in the LHRH pulse generator, indicating either a limitation of this PERF technique or that alterations in AP response to LHRH after CAST may be responsible for the increase in LH levels and pulsatility after CAST. (Supported by NIH HD 11362).

431.9

CELLULAR LEVELS OF mRNA ENCODING GONADOTROPIN RELEASING HORMONE (GnRH) IN INTACT, CASTRATED AND HYPERPROLACTINEMIC MALE RATS. M. Selmanoff, C. Shu*, S.L. Petersen, C.A. Barraclough and R.T. Zoeller, Dept. of Physiol., Univ. of Maryland, Sch. of Medicine, Baltimore, MD 21201 and LNC, NINCDS, Bethesda, MD 20892.

We quantitated single cell mRNA levels in brains of intact and castrated rats and in castrated rats bearing the 7315a PRL-secreting tumor. Nine days postcastration, rats were decapitated and 12μm sections prepared from the diagonal band of Broca through the medial preoptic nucleus (MPN). Sections were hybridized with an ^{35}S-labelled 48-base oligonucleotide probe. A single section in the region of the MPN was chosen from each brain and quantitated using darkfield optics in a Bioquant Image Analysis System IV. The average number of cells labelled/section was 8.9 ±0.5 (n=21) and the average cellular labelling intensity was 50 times background.

	Intact	Castrated	Castrated, tumor-bearing
Serum LH (ng/ml)	61 ± 8	634 ± 44	277 ± 84
Grain density	114.2 ± 5.7	130.4 ± 2.5	121.2 ± 6.1

The grain density of labelled cells was significantly increased postcastration and this increase was suppressed by the presence of a PRL-secreting tumor. The data indicate that testicular steroids and PRL affect steady state GnRH mRNA levels in cells residing in the region of the MPN. Supported by NIH grants HD-21351 and HD-02138.

431.10

DO GONADOTROPIN RELEASING HORMONE (GNRH) OR DOPAMINERGIC NEURONS IN THE SHEEP CONTAIN ESTRADIOL RECEPTORS? F.J. Karsch* and M.N. Lehman (Spon: G. Blaha). Develop. & Reprod. Biol., Univ. Mich., Ann Arbor, MI; Dept. Anat. & Cell Biol., U. Cincinnati Coll. Med., Cincinnati, OH.

Estradiol exerts a major feedback influence upon GnRH neurons which comprise a pulse generator controlling pituitary gonadotropin secretion. Using double label immunocytochemistry with a monoclonal antibody against the estradiol receptor (ER) (H222, Abbott Laboratories) we determined whether sheep GnRH neurons, or tyrosine hydroxylase (TH)-positive neurons which may be afferent to them, contain ER. Adult Suffolk ewes, either intact (n=2), ovariectomized (OVX) (n=2), or OVX with estradiol implants (OVX+E) (n=1), were perfused during anestrus with 4% paraformaldehyde. An immunoperoxidase/immunofluorescence procedure was used to visualize both ER and either GnRH or TH in the same preoptic and hypothalamic sections. Cells with nuclei immunostained for ER were concentrated in the medial preoptic area, periventricular anterior hypothalamus, and the paraventricular, ventromedial hypothalamic nucleus, and arcuate nuclei. Thus far, we have found no GnRH neurons which also contain immunoreactive ER although preoptic GnRH neurons were frequently in close proximity to ER-positive cells. We did find a small number of TH neurons which contained ER-positive nuclei in the periventricular region of the anterior hypothalamus (A14) and in the arcuate nucleus (A12). No differences between intact and OVX ewes were apparent in the distribution of cells with ER-positive nuclei. These results suggest that in sheep estradiol may not act directly upon GnRH neurons but upon cells which project to them. [Supported by NIH HD 18337 (FJK) and HD 21968 (MNL)]

431.11

ESTRADIOL INHIBITS SEPTAL NEURONS PROJECTING TO MEDIAN EMINENCE S.D. Donevan and A.V. Ferguson. Dept. of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The perikarya of luteinizing hormone releasing hormone (LHRH) immunoreactive neurons that project to the median eminence are located in a continuum from the septal region through to the anterior hypothalamus. Recordings have been obtained from neurons in the septal-preoptic area that could be antidromically identified as projecting to the median eminence and it has been suggested that these identified neurons are LHRH neurons. In the female rat, putative LHRH-containing preoptic neurons which project to the median eminence are influenced by systemic estrogen, suggesting an inhibitory role for this steroid in the control of LHRH secretion. The present study examines, in the male rat, the effect gonadal steroids on medial septal neurons identified as projecting to the median eminence.

In anesthetized male rats, extracellular recordings were obtained from 32 medial septal neurons antidromically identifed as projecting to the median eminence. Antidromic action potentials were followed by a period of inhibition (100 ms), which frequently persisted (83%) when the stimulus delivered to the median eminence was below threshold for antidromic activation. The spontaneous activity of 50% of identified septal neurons tested (n=10) was decreased following intravenous injection of 17β-estradiol (0.5-2 μg). This supports an inhibitory role for 17β-estradiol in the regulation of LHRH secretion in the male rat.

Supported by MRC of Canada

431.12

TESTOSTERONE, NOT INHIBIN, IS THE MAJOR SUPPRESSOR OF FSH SECRETION IN THE ADULT MALE RAT. M.D. Culler* and A. Negro-Vilar (SPON: W. Wetsel). Reprod. Neuroendo. Sect., LMIN, NIEHS, NIH, Research Triangle Park, NC 27709.

We have previously observed that immuno-neutralization of endogenous inhibin (I) in unanesthetized rats results in a dramatic elevation in plasma FSH in the female but not in the adult male. In order to further test the role and interaction of I and testosterone (T) in regulating FSH secretion, adult male rats were injected ip. either with ethylene dimethane sulphonate (EDS), a compound that selectively destroys the Leydig cells, or with vehicle alone. After 7 days, both groups of rats were injected iv. either with anti-I serum MC-4 or with normal sheep serum (NSS). Blood samples withdrawn 8 hrs later from the EDS-treated rats contained no detectable T as compared with a mean level of 1.34 ± 0.26 ng/ml in the vehicle-treated rats. Plasma FSH and LH levels in the EDS-treated rats were approx. 3- and 15-fold higher, respectively, than in the vehicle-treated controls. Anti-I serum treatment caused no significant changes in either gonadotropin as compared with NSS-treated rats. These results indicate that T, not I, is the major suppressor of plasma gonadotropin secretion in the adult male rat.

432.1

IMMUNOPURIFICATION OF BUTYRYLCHOLINESTERASE FROM CHICKEN SERUM: MOLECULAR PROPERTIES AND COMPARISON WITH THE MUSCLE ENZYME. K.W.K. Tsim*, W.R. Randall* and E.A. Barnard*. (SPON: M. Smith). MRC Molecular Neurobiology Unit, MRC Centre, Cambridge CB2 2QH, U.K.

Chicken serum butyrylcholinesterase (BuChE;EC 3.1.1.8) was purified sequentially on an anti-BuChE immunoaffinity column and a N-methylacridinium column. The purified enzyme has a specific activity of 125 U/mg of protein on acetylcholine as substrate, representing over 13,000-fold purification. The purified serum BuChE contains one type of catalytic subunit, apparent M_r 76,000, which can be labeled [^{3}H] diisopropylfluorophosphate; that reaction is blocked by a pretreatment with tetraisopropylpyrophosphoramide. The subunit size of serum BuChE is 4,000 daltons larger than that of chicken muscle BuChE; this difference appears to be in the carbohydrate content, since N-glycanase digestion of both enzymes leads to an identical size in SDS-PAGE. Over 95% of the purified serum BuChE is in the form of the globular tetramer which sediments at 11 S. This tetrameric BuChE consists of two disulphide-bridged dimers, each showing in SDS-PAGE an apparent M_r of 150,000 in the non-reduced state.

432.2

PHYSOSTIGMINE PROTECTS RAT HIPPOCAMPAL SLICE FROM IRREVERSIBLE DIISOPROPYL PHOSPHOROFLUORIDATE (DFP) INDUCED EPILEPTIFORM BURSTING. M. Abou-Donia, L.S. Jones, D. Lapadula*, and D.V. Lewis. Depts. of Pharmacology and Pediatrics, Duke Univ. Med. Ctr., Durham, NC 27710.

We have shown previously that DFP produces rapid, irreversible bursting in the CA3 subfield of hippocampal slices, while having little effect on the response of CA1 (Soc. Neur. Abs. 13:1354). Results with atropine suggested that the effects were partially mediated through the muscarinic cholinergic receptor; further evidence that DFP effects were cholinergic was needed. Using 625 μ slices from adult, male Sprague-Dawley rats cut in the usual manner, we have examined the ability of physostigmine (10 uM), a reversible AChE blocker, to protect the slice from the effects of DFP. Results indicate that, though the slices become somewhat hyperexcitable in the presence of physostigmine, the effect is reversible with washing. Further, when DFP is added to slices that are already bathed in physostigmine, the slices do not burst as they do in DFP alone, and the hyperexcitability is still reversible, which is not seen with DFP alone. These results point to cholinergic system activation as the primary mechanism for DFP-induced bursting in CA3.

(Supported in part by NIEHS Grant No. ES02717).

432.3

NEUROCHEMICAL DIFFERENCES IN THE CAT VISUAL SYSTEM AFTER ANTICHOLINESTERASE AGENTS. A.W. Kirby, A.T. Townsend*, R.G. Stafford* and T.H. Harding*. U.S. Army Aeromedical Research Lab., P.O. Box 577, Ft. Rucker, AL 36362-5292.

We reported previously that the anticholinesterase agents diisopropylfluorophosphate (DFP) (Brain Res. 325: 357), and physostigmine (Science 221:1076) have similar effects on the cat visual evoked response (VER). Recent retinal release studies suggest that DFP has a direct effect on neuronal membranes (Soc. Neurosci. Abstr. 13:1057). We report here retinal and cortical neurochemical differences following administration of the two agents.

Retina and visual cortex were removed from anesthetized and paralyzed adult cats before and after physostigmine, analyzed with high performance liquid chromatography, and compared to earlier results with DFP.

Previous results with DFP suggest that cortical dopamine (DA) turnover (avg. inc. 117%) and GABA (avg. dec. 25%), as well as retinal DA (avg. dec. 28%) were linked to VER changes. Following physostigmine, there is no consistent change in DA turnover, and GABA increases in cortex (avg. 96%). Retinal DA decreases (avg. 15%). Other differences are seen in cortical glycine, aspartate (asp), epinephrine, and norepinephrine, as well as retinal asp, glutamate, and GABA.

Since the VER and cholinesterase changes are similar, the neurochemical differences following the two agents are difficult to explain. Since both have cholinergic effects, membrane effects of DFP might account for the differences.

432.4

COMPARISON OF *IN VITRO* ANTICHOLINESTERASE PROPERTIES OF PHYSOSTIGMINE AND PHYSOSTIGMINE DERIVATIVES. J.R. Atack, Q.-S. Yu, A. Brossi and S.I. Rapoport (SPON: J. Noronha). Labs of Neuroscience, NIA and Analytical Chemistry, NIDDK, NIH, Bethesda, MD 20892.

Physostigmine (eserine), which is a carbamate ester alkaloid and a potent inhibitor of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), has recently been used in the experimental treatment of Alzheimer's disease with largely disappointing results. However, since physostigmine has a plasma half-life of less than 30 min., physostigmine derivatives with longer half-lifes may be more clinically useful than physostigmine itself. As a first step in the identification of such compounds, we synthesized two series of physostigmine analogues which were either carbamate- or N(1)-substituted derivatives. The *in vitro* potencies (IC_{50}) of these 20 compounds against human brain and erythrocyte AChE and human brain and plasma BChE were compared to that of physostigmine. For each compound, the IC_{50} against human brain and erythrocyte AChE was very similar, although the IC_{50} of human plasma BChE was consistently 5-15-fold less than for human brain BChE, suggesting that with respect to inhibitor susceptibility, human AChE is similar throughout the body whereas BChE is not. With respect to human AChE, 6 compounds (octyl-, butyl-, benzyl- and N-phenyl-carbamoyl eseroline and N(1)-nor- and N(1)-allyl-physostigmine) had IC_{50}'s (ranging from 15 ± 1 to $37\pm7\times10^{-9}$M) similar to the IC_{50} of physostigmine ($31\pm8\times10^{-9}$M). Of these compounds, only N-phenyl carbamoyl eseroline was a relatively selective inhibitor of AChE rather than BChE (IC_{50} vs. human brain AChE and BChE $=36\pm3$ and $2500\pm1100\times10^{-9}$M, respectively). Eseroline, which is presumably the major metabolite of physostigmine, was a very poor anticholinesterase.

432.5

TETRAHYDROAMINOACRIDINE (THA) EFFECTS ON BEHAVIOR IN RODENTS. D. S. Chapin* and J. A. Nielsen (SPON: L. K. Torgersen). Pfizer Central Research, Groton, CT.

Senile dementia of the Alzheimer type (SDAT) is a neurodegenerative brain disorder characterized by severe memory impairment. A decrease in neurochemical markers associated with cholinergic neurons has been found in SDAT patients. This suggests that cholinergic neurotransmission may be compromised in SDAT and that pharmacotherapy to enhance cholinergic function may be of benefit. THA, a potent cholinesterase inhibitor, produced symptomatic improvement in a limited trial with SDAT patients. To determine if the clinical findings with THA would be predicted from standard animal testing procedures, we have assessed the effect of THA in rodent models and estimated the therapeutic ratio by evaluating autonomic and behavioral side effects.

THA reversed scopolamine-induced amnesia in a T-maze procedure after i.p. (3.2 mg/kg) and p.o. (32 mg/kg) treatment in rats. The drug also improved the performance of mice in a passive avoidance model of memory function after 3.2 mg/kg, i.p. THA produced hypolocomotion at 5.6 mg/kg i.p., tremors and salivation at 17.8 mg/kg i.p. All of these side effects were prevented by scopolamine, a general muscarinic receptor antagonist, while the peripheral muscarinic antagonist glycopyrrolate inhibited only salivation.

These results suggest that THA is active in rodent models of cognition. The side effects of THA observed in rodents probably have both peripheral and central nervous system components.

432.6

STRUCTURE ACTIVITY RELATIONSHIPS FOR A SERIES OF (±)-9-AMINO-1,2,3,4-TETRAHYDROACRIDIN-1-OL (HP 029) ANALOGS. F.P. Huger, G. Bores*, G. Shutske* and D.B. Ellis*. Departments of Biological and Chemical Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J. 08876.

Tacrine, a potent cholinesterase inhibitor, has been reported to benefit Alzheimer's disease patients. However, side effect liability may be a limiting factor. A number of HP 029 analogs were synthesized and tested for inhibition of acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and biogenic amine uptake. AChE was measured in rat striatal homogenates by a modification of the Ellman method. BChE was determined similarly, except butyrylthiocholine was used as the substrate and human serum as a source of enzyme. Biogenic amine transport was measured in synaptosomal preparations from rat brain.

Whereas tacrine and the 1-hydroxyl compound (HP 029) are more potent as inhibitors of BChE than AChE, halogen substitution at the 6-position of the aromatic ring increased the potency for inhibition of AChE and reversed the selectivity of inhibition (i.e., AChE>BChE). Propyl or benzyl substitution at the 9-amino nitrogen decreased the effect on AChE. A most interesting finding was that certain benzyl-substituted analogs were also potent inhibitors of biogenic amine uptake (NE>DA>5HT). The inhibition of biogenic amine uptake by the benzyl-substituted compounds occurred at lower concentrations than the reported ion channel and NE release effects of tacrine. Compounds with combined cholinomimetic and adrenergic activity may show better efficacy in patients with multiple neurotransmitter deficits.

432.7

EFFECT OF THA ON ACH RELEASE IN ANIMALS WITH CHOLINERGIC LESIONS. P.E. Potter and S. Nitta*. Department of Anesthesiology, Albert Einstein College of Medicine, Montefiore Hospital, 111 E. 210th St., Bronx, N.Y. 10467

It has been reported that tetrahydroaminoacridine (THA), which decreases acetylcholine (ACh) release from normal brain, may enhance ACh release from brain slices of patients with Alzheimer's disease. We have lesioned cholinergic neurons in rat cortex by bilateral injection of 100 nmoles quinolinic acid into the substantia innominata, or lesioned hippocampal cholinergic terminals by intraventricular administration of 2 nmoles of ethylcholine mustard aziridinium (AF64A). The effects of THA (5 x 10^{-5} M), physostigmine (10^{-5} M), and LF-14 ([3,3-dimethyl-1-(4-amino-3-pyridyl) urea]; 5 x 10^{-5} M) were compared on electrically stimulated (2 Hz, 2 min) release of ^{3}H-ACh from hippocampal and cortical slices taken from control and lesioned rats. The slices were stimulated 3 times. Drugs were added between S_2 and S_3, and results are expressed as the S_3/S_2 ratio for fractional release. THA, like physostigmine, significantly decreased the S_3/S_2 ratio in hippocampus (Control= 0.97±0.03; THA= 0.75±0.13; physostigmine= 0.65±0.07) and cortex (Control= 0.90±0.06; THA= 0.37 ±0.09, physostigmine= 0.46±0.02). LF-14, a 3,4-diaminopyridine derivative, caused a large increase in ACh release (S_3/S_2 ratio: hippocampus= 4.51±1.35; cortex= 1.75 ±0.18). There was no significant difference in any of these values in slices from rats lesioned with AF64A or with quinolinic acid.

432.8

EFFECT OF CHOLINE ON CATECHOLAMINES SECRETION FROM THE ISOLATED PERFUSED RAT ADRENAL GLAND. İ.H.Ulus,L.Büyükuysal*and B.K.Kıran*.Dept. of Pharmacology, Uludağ University Medical Faculty,Bursa, TURKEY.

Male rats (250-320 g) were anesthetized with ether. The abdomen was opened by a mid-line incision, and the left adrenal gland and surrounding area were exposed. A cannula (PE 10) was inserted into the adrenal vein after all the small branches of the adrenal vein were ligated. A small slit was made into the adrenal cortex just opposite the entrance of the adrenal vein. Then the adrenal gland, along with the tied vessels an the cannula, was carefully removed from the animals. The gland was perfused with a buffered physiological solution (pH 7.4) at a rate about 0.4 ml/min. The solution was continously bubbled with 95% O_2+ 5% $CO_2$and was kept at 37o in a water bath. Perfusates were collected and assayed for catecholamines (CA). Addition of increasing concentrations (0.5-32 mM) of choline into the superfusion medium evoked CA secretion concentration related manner. The concentrations of choline lower than 0.5 mM failed to induce a detectable CA release into the medium. The secretion of CA induced by 5 mM of choline was blocked either by mecamylamine (1-10 µM) or by Hemicholium-3 (1 µM). Atropine (1-5 µM) was failed to alter CA secretion induced by 5 mM choline. These results show that choline has ability to induce CA secretion from the adrenal gland in vitro conditions, and the activation of nicotinic receptors via presynaptic mechanisms apparently involves in its actions.

432.9

MEASUREMENT OF ACETYLCHOLINE BY HPLC WITH ELECTROCHEMICAL DETECTION AND EFFECTS OF DOPAMINERGIC AND CHOLINERGIC AGENTS ON ACETYLCHOLINE LEVELS. F.P. Bymaster* and D.T. Wong (Spon: L. Truex). Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

Acetylcholine (ACH) and choline (CH) are difficult to measure in tissues. Potter et al. (1983) and Bymaster et al. (1985) have reported methods for measuring ACH and CH using HPLC with electrochemical detection. CH and ACH in tissue extracts were separated, and choline oxidase and acetylcholinesterase were mixed with the eluate. The hydrogen peroxide formed was quantitatively detected. A new method allows enzyme immobilization on a column, resulting in increased sensitivity. Using this method, rats treated with the dopamine agonists pergolide (300 µg/kg), quinpirole (300 µg/kg) and LY163502 (50 µg/kg) had levels of ACH increased in striatum to 180, 212 and 228%, respectively. Dopamine antagonists haloperidol (0.3 mg/kg) and fluphenazine (1 mg/kg) lowered ACH levels to 62 and 64%, respectively. Oxotremorine (0.25 mg/kg), a cholinergic agonist, raised ACH levels in to 181%. Physostigmine (0.5 mg/kg), an acetylcholinesterase inhibitor, raised ACH levels to 202%. Cholinergic antagonists scopolamine (0.6 mg/kg) and atropine (10 mg/kg) lowered ACH levels to 56 and 23%, respectively. Thus it appears that cholinergic and dopaminergic agonists decrease ACH turnover, whereas, the antagonists increase turnover.

432.10

REGULATION OF CHOLINE ACETYLTRANSFERASE (ChAT) IN CULTURED EMBRYONIC CHICK SPINAL CORD NEURONS. C.L. Weill. Dept. of Neurology and Anatomy, Louisiana State Univ. Med. Center, New Orleans, LA 70112.

Choline acetyltransferase is induced by cAMP derivatives but not by 8-Br-cGMP in dissociated fetal mouse spinal cord-DRG cultures (J. Neurochem. **41**:1349, 1983). A re-examination of ChAT induction in cultured chick embryo spinal cord neurons revealed that ChAT was induced by cAMP derivatives and db-cGMP but not by 8-Br-cGMP. Induction by both db-cAMP and db-cGMP was dose dependent from 4 uM to 4 mM. The maximum induction effected by db-cGMP was consistently less than observed for db-cAMP, while the time course for induction by db-cGMP was different from that for db-cAMP; ChAT was maximal after 4-5 days and 2-3 days with db-cGMP and db-cAMP respectively. ChAT induction by both analogs was inhibited by both cycloheximide and actinomycin D suggesting a requirement for protein synthesis and RNA transcription. ChAT was also induced by chlorophenylthio-cAMP, an analog that is 100x more potent than cAMP at activating protein kinase A. Further, a dose dependent induction was effected by factors in embryonic muscle cytosol. No dependence on steroid hormones was observed, and insulin, somatostatin, atrial natriuretic factor, the phorbol ester, TPA, and nitroprusside were not inductive.

432.11

EFFECTS OF LANTHANUM, HIGH POTASSIUM AND α-LATROTOXIN ON INTRACELLULAR CALCIUM AND ACETYLCHOLINE IN SYNAPTOSOMES. H. W. Scheer*,1, L. Rosenthal*,2 and B. Collier3. Depts. of Pharmacology, Université de Montréal1 and McGill University3, Montreal, CANADA; Istituto Scientifico San Raffaele2, Milano, ITALY.

At the neuromuscular junction, La^{3+} has a dual effect on acetylcholine (ACh) release:basal release is augmented, stimulated release is blocked. To determine effects of La^{3+} in the CNS, rat cortex synaptosomal preparations were exposed for 10 min to La^{3+} (100µM), high K^+ (35mM) or the neurosecretagogue α-Latrotoxin (α-LTx; 2nM). ACh release and tissue contents were measured radioenzymatically; free intrasynaptosomal [Ca^{2+}] was measured by FURA-2 fluorescence. While basal [Ca^{2+}] were largely unaltered by La^{3+}, the increase in intrasynaptosomal Ca^{2+} elicited by high K^+ or α-LTx (1.6 and 4.8-fold increase resp.) was completely blocked by La^{3+}. Similarly, the increase of ACh released into medium following a 10 min incubation in high K^+ or α-LTx (2.0 and 2.8-fold increase resp.) was blocked by La^{3+}. The reduction in synaptosomal ACh content afforded by K^+ and αLTx (40% and 70% decrease resp.) was inhibited by La^{3+}. In contrast to evoked release, La^{3+} had no marked effects on either basal ACh release or contents. These results indicate that while in non-stimulated cortex synaptosomes La^{3+} does not greatly affect either ACh release or contents, La^{3+} does inhibit changes in ACh and intrasynaptosomal Ca^{2+} induced by high K^+ or α-LTx.

432.12

EFFECT OF INTERLEUKIN-2 (IL-2) ON ACETYLCHOLINE RELEASE IN RAT BRAIN: CORRELATION WITH IL-2 BINDING SITES. D.M. Araujo, P.A. Lapchak, B. Collier, and R.Quirion. Douglas Hospital Res. Ctr. and McGill Univ., Montreal, Canada.

In the present work, we characterized the distribution of IL-2-like immunoreactivity (IR) and IL-2 binding sites in rat brain, both in the adult and during ontogeny. In addition. we tested the effects of IL-2 on acetylcholine (ACh) release from brain slices. Radioimmunoassay analysis of tissue extracts showed that IL-2-like IR is present in hippocampus, striatum, and frontal cortex (0.70, 0.62, 0.12, ng/mg tissue, respectively). Using in vitro autoradiography and membrane binding techniques, specific IL-2 binding sites were detected in hippocampus (Bmax= 0.40 fmol/mg prot; Kd= 20 pM); specific binding was not apparent in either striatum or frontal cortex. In hippocampal slices, IL-2 caused a concentration-dependent inhibition of evoked ACh release; this IL-2 effect was enhanced in slices from kainate-lesioned hippocampi. In contrast, IL-2 did not significantly alter the release of ACh from either striatal or cortical slices. Furthermore, the IL-2-induced reduction of hippocampal ACh release appears to be mediated by a specific IL-2 receptor, since IL-1 (1 nM-10 uM) did not affect this measure. In summary, endogenous IL-2-like IR and specific IL-2 receptor sites are present in rat brain; activation of these sites by endogenous IL-2 may inhibit ACh release from certain brain areas. (Supported by MRC, Canada and FRSQ and FCAR, Quebec).

432.13

DIFLUNISAL ACTION ON INTESTINAL SMOOTH MUSCLE FROM THE RAT. E. Gijón*, E. Castillejos*, and X. García*.(SPON: B. Ortega-Corona). Dept. of Physiol. Sch. of Med. Universidad Nacional Autónoma de México, Ap. P. 70-250, México, D.F. 04510. MEXICO.

It has been reported that diflunisal (5-(2,4-difluorophenyl) salicilic acid) inhibits mitochondrial oxidative phosphorylation (McDougal, P., Markham, A., Cameron, I., and Sweetman, A.J. Biochem. Pharm., 32:2595-2598, 1983) and as a consecuence of the above, it induces a decrease of the mitochondrial membrane potential and therefore the release of intramitochondrial calcium and also that diflunisal behaves as an ionophore molecule (Chávez, E., Bravo, C., Gil, H.A., and Reyes, P.A. Life Sci. 37(16):1491-1498, 1985). Segments of intestine from 125 g male Wistar rat were prepared for isometric recording. It is shown that diflunisal, from Merck Sharp and Dohme de México, causes a contraction followed by a pronounced relaxation in intestinal muscle. In addition this report presents evidence that diflunisal modifies acetylcholine induced contraction. When diflunisal is applied during an acetylcholine contraction their effects are magnified. Diflunisal actions might be promoted by the release of the accumulated calcium of the mitochondria. These results also suggest an ionophoretic-like action at the muscle membrane, while a presynaptic action is not discarded, or an interaction with the acetylcholine receptor.

432.14

ENDOGENOUS ACETYLCHOLINE (ACh) RELEASE IN VIVO: MEASUREMENT BY INTRACEREBRAL MICRODIALYSIS AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS). M.R. Marien and J.W. Richard*, Douglas Hospital Research Center, Verdun, Quebec, Canada.

Male SD rats (275-325g) were anesthetized with urethane (1.2 g/kg) and implanted with a microdialysis probe (BAS-Carnegie Medicin) terminating in the striatum. Under anesthesia, probes were perfused with Mg2+-free Ringer's solution for 8 hours, during which time drugs were administered either i.p. or via the probe. Perfusate samples (20 min, 46 ul) were mixed with internal standard (deuterated ACh) and processed for GC-MS analysis of ACh according to Wood & Peloquin (Neuropharm. 21: 349, 1982), with modifications. Assay sensitivity was 1-2 pmole ACh.

Dialyzable levels of striatal ACh (30-70 pmoles/20 min, in the presence of 75 uM physostigmine, not corrected for probe efficiencies of 10-26 %) were: (1) measureable only in the presence of physostigmine ($\geq$ 10 uM), (2) reduced by Ca2+-free Ringer's, tetrodotoxin (0.1 uM) and vesamicol (300 nM or 5 mg/kg i.p.), and (3) increased by K+ (100 mM) and haloperidol (0.75 mg/kg i.p.). Results are consistent with the known pharmacology of the striatal cholinergic system, and demonstrate the sensitivity and validity of this method for monitoring striatal ACh release in vivo. (Supported by the Douglas Hospital Research Centre).

432.15

QUANTITATIVE CHOLINERGIC EVALUATION OF SPINAL CORD IN ORGANOTYPIC CULTURES. Judith Friend, Changiz Geula, Shinji Ishimoto, and John R. Delfs. Department of Neurology, Harvard Medical School, Arnold Pain Center, New England Deaconess Hospital, and Dana Institute, Beth Israel Hospital, Boston, MA 02215

Anatomical and histological integrity of transverse sections of spinal cord in organotypic roller tube culture has been reported previously (Delfs and Saroff, Soc. Neurosci. Abst. 12:386, 1986). The purpose of this work was to determine whether cholinergic neuron morphology and biochemistry can be quantitated in these cultures. Cultures were studied after three weeks in vitro. For analysis of neuron size, intact cultures were stained for acetylcholinesterase (AChE) activity and the two-dimensional area of AChE-positive ventral horn neurons measured. Areas ranged up to 1635 with an average of 245 +/- 104 square microns, sizes comparable to reported in vivo values. Biochemically, pooled cultures showed choline acetyltransferase activity of 0.47 +/- 0.19 and AChE activity of 12.2 +/- 4.9 uMol/min/g protein (n=7), values comparable to those reported for neonatal rat spinal cord. These cultures can be used to study factors affecting ventral horn cholinergic neurons in vitro.

432.16

EFFECTS OF CHOLINOACTIVE COMPOUNDS ON AXIAL MUSCLE OF *HAEMONCHUS CONTORTUS* AND *BRUGIA PAHANGI*. M. A. Travis and D. P. Thompson. Parasitology Research, The Upjohn Company, Kalamazoo, MI 49001.

The cholinergic receptor on nematode axial muscle represents an important site for anthelmintic action. Acetylcholine is the major excitatory transmitter at neuromuscular junctions in nematodes, and two important commercial anthelmintics, levamisole and pyrantel, mimic the actions of acetylcholine in several physiological assays using *Ascaris suum* (Coles, G.C., *et al.* Gen. Pharmacol., 6:309-313, 1975; Harrow, I.D. and Gration, K.A.F., Pestic. Sci., 16:662-672, 1985). To determine the impact of cholinergic stimulation and inhibition on a nematode of importance to veterinary medicine, the effects of several nicotinic and muscarinic (ant)agonists on isolated axial muscle of adult female *Haemonchus contortus* (H.c.) were measured using a modified suction electrode-balance beam system. For comparative purposes, analogous studies were conducted using muscle strips obtained from adult female *Brugia pahangi* (B.p.), an accessible filarial nematode that is cultured in gerbils. Results of these studies support the following conclusions: (1) nicotinic agonists stimulate a spastic contraction and sustained paralysis in H.c., while in B.p., they stimulate a biphasic response (contraction, followed by flaccid paralysis); (2) neither parasite is influenced by muscarinic agonists or antagonists; (3) nicotinic antagonists exert no independent effect on parasite muscle tension, nor do they influence responses to nACh agonists; (4) B.p. desensitize to nicotinic stimulation; (5) cross-desensitization occurs among nicotine, levamisole and pyrantel. These results suggest that potentially important differences exist between nematode and mammalian nACh receptors.

432.17

CHOLINERGIC MUTANTS OF THE NEMATODE C. Elegans. James B. Rand, Aixa Alfonso-Pizarro*, and Carl D. Johnson. Dept. of Zoology, Univ. of Wisconsin, Madison, WI 53706, and Cambridge NeuroScience Research, Cambridge, MA 02139.

In order to identify genes affecting cholinergic metabolism and regulation, we have been isolating mutations that confer resistance to cholinesterase inhibitors such as the pesticide Aldicarb. Previous studies have shown that at least 12 different genes can mutate to give resistance. One of these genes is the cha-1-unc-17 complex, which we have shown to be the structural gene for choline acetyltransferase (the acetylcholine biosynthetic enzyme). Other resistance loci include unc-1, unc-10, unc-11, unc-13, unc-18, unc-32, unc-36, unc-63, unc-64, unc-65, and lan-5. We believe that many of these genes may affect acetylcholine metabolism, release, or function. We have isolated more than 400 independent spontaneious Aldicarb-resistant mutants from several starting strains known to contain active transposons; we expect that many of these mutants result from transposon insertions. Many of these mutants are severely uncoordinated or paralyzed, while others have only slight behavioral defects. Mapping experiments indicate that this set includes alleles of most or all of the known resistance loci (including at least 7 new alleles of cha-1-unc-17), plus at least one previously uncharacterized resistance locus on Linkage Group IV. (Supported by a Ford Foundation Postdoctoral Fellowship to A.A.-P. and research grants from NIGMS and MDA to J.B.R.)

432.18

AFFINITY-LABELLING AND PARTIAL CHARACTERIZATION OF THE CHOLINE CARRIER FROM TORPEDO ELECTROMOTOR NERVE TERMINALS, USING ^{3}H-CHOLINE MUSTARD. R.Jane Rylett and V.P.Whittaker Dept. Physiology, University of Western Ontario, London, Canada and Arbeitsgruppe Neurochemie, Max-Planck-Institut fur Biophys.Chemie, Gottingen, FR Germany.

Isolation and characterization of the high-affinity choline carrier is an important step towards understanding the organization of the cholinergic nerve terminal at the molecular level. The choline transporter in presynaptic plasma membranes of electric organ of Torpedo marmorata was radiolabelled with ^{3}H-choline mustard arizidinium ion, a known irreversible inhibitor of choline transport in rat brain synaptosomes. Membrane proteins were solubilized from EDTA-washed synaptic membranes with 0.6% CHAPS, then fractionated on a calibrated column of Ultrogel ACA44. The ^{3}H-labelled protein(s) were recovered as a peak with Stoke's radius of about 4.3 nm. Samples from the gel permeation chromatography were separated by equilibrium centrifugation on a 5-20% linear sucrose gradient; the ^{3}H-labelled protein was collected near the top of the gradient and had a sedimentation coefficient of about 5.5 S. Estimation of the molecular weight of the native protein-detergent-lipid complex was about 100 kDa. Separation of the ^{3}H-labelled protein by SDS-PAGE indicated that the majority of the ^{3}H was associated with a polypeptide subunit at 40-42 kDa. Labelling of the protein(s) was inhibited by hemicholinium. (Supported by the Medical Research Council of Canada, the Max-Planck Geselleschaft and EMBO).

432.19

DIFFERENCES IN OPEN FIELD BEHAVIOR IN RECOMBINANT INBRED STRAINS OF MICE. J.I. Nurnberger, Jr., J.R. Simon and J.N. Hingtgen. Inst. Psychiat. Res. and Program in Medical Neurobiol., Depts. of Psychiat. and Biochem., Indiana U. School of Med., Indianapolis, IN 46223.

Studies of recombinant inbred strains of mice may provide useful genetic data on potential relationships between cholinergic neurochemistry and behavior. Initial investigations in this laboratory on the progenitor strains, DBA/2J and C57BL/6J, indicated that DBA's have lower cholinergic activity than C57's in hippocampus and striatum (though the DBA's have higher CAT activity in striatum). DBA's also show a lower number of total crossings and center crossings than C57's in open field behavior. The cholinergic antagonist, scopolamine, and the agonist, arecoline, produced differential behavioral effects in these mice. In the present study, significant differences in open field behavior (total crossings, center crossings and rearings) were obtained when six recombinant inbred strains (phenotypic offspring of DBA and C57) were observed. Open field behaviors in these strains following arecoline and scopolamine are also being studied. In addition, high affinity choline uptake and choline acetyltransferase activity will be measured in specific brain areas of these mice. (Supported in part by Indiana Department of Mental Health grant.)

432.20

EPIDERMAL GROWTH FACTOR (EGF) BINDING SITES IN ADULT RAT BRAIN AND PITUITARY GLAND. AN IN VITRO AUTORADIOGRAPHIC STUDY. J.-G. Chabot, D.M. Araujo and R. Quirion. Douglas Hosp. Res. Ctr., Dept. of Psychiatry, McGill University, Verdun, Canada.

Recent studies have indicated that EGF may act as neurotrophic and/or neuromodulator substance in mammalian brain. Using an in vitro receptor autoradiographic method, we have studied the distribution of EGF binding sites in the rat brain and pituitary gland. Tissue sections were incubated with ^{125}I-EGF (200 pM) in the absence or presence of an excess of unlabeled EGF (200 nM) and juxtaposed against tritium-sensitive film. Autoradiographic data clearly demonstrate the discrete distribution of EGF sites in rat brain. ^{125}I-EGF binding sites are observed in several brain areas, such as cerebral cortex, striatum, hippocampal formation, certain hypothalamic nuclei, medial geniculate nucleus, ventral tegmental area, substantia nigra pars compacta and cerebellum. In the pituitary gland, EGF receptor sites are restricted to the pars distalis. Thus, EGF binding sites are present in rat brain and pituitary gland suggesting the possible involvement of this factor in the maintenance of normal CNS functions (see Quirion et al., this meeting). (Supported by MRC, Canada and FRSQ, Quebec).

432.21

GROWTH FACTOR RECEPTORS IN RAT BRAIN: LOCALIZATION AND INTERACTION WITH THE CHOLINERGIC SYSTEM. R. Quirion, D. M. Araujo, P. A. Lapchak, J.-G. Chabot, and B. Collier. Douglas Hosp. Res. Ctr. & McGill Univ., Montreal, Canada.

The present study characterized the binding of several growth factors (GFs) including insulin-like GF (IGF-1), epidermal GF (EGF), and nerve GF (NGF). Using in vitro autoradiography and membrane binding assays, we studied the ontogenetic development of GF receptor sites in the rat brain. In addition, we tested the effects of these GFs on acetylcholine (ACh) release from rat brain slices.

The results show that there is an extensive distribution of IGF-1 receptor sites in rat brain, and that these sites undergoredistribution during development and maturation. EGF sites appear to be mostly exposed during postnatal development early on (P1). In slices of hippocampus, which is highly enriched with IGF-1 sites, and some EGF sites, IGF-1 and EGF reduced the potassium-evoked (25 mM) release of ACh; this effect was evident in slices from adult, but not immature, hippocampus. In contrast, although specific NGF binding sites are present in adult rat hippocampus, NGF did not alter ACh release in either adult or immature rat hippocampus. Thus, it appears that in addition to their well-known trophic effects, IGF-1 and EGF may function as neuromodulators of ACh release in the adult rat brain. (Supported by MRC, Canada and FRSQ and FCAR, Quebec)

CATECHOLAMINES V

433.1

AN AGE STUDY ON THE LEVELS OF CYSTEINYLDOPAMINE AND RELATED ADDUCTS IN THE HUMAN BRAIN B. Fornstedt*, (SPON:Liljequist) Department of Pharmacology, University of Göteborg, BOX 33031, S-400 33 Göteborg, Sweden.

The cellular mechanism behind the selective degeneration of neuromelanin containing neurons in brain disorders as Parkinson's disease has been a matter of speculation for many scientists. During nonpathologic aging there is a similar but much less pronounced loss of neurons. It is well known that catecholamines undergo autoxidation to semiquinones and quinones and it is believed that further oxidation of these products with a subsequent polymerization gives rise to neuromelanin. The electron deficient quinones react readily with nucleophilic compounds, especially thiol containing substances as cysteine and glutathione which are highly concentrated in the cytosol. The higher vulnerability of catecholaminergic neurons can be explained by the reactivity of the quinones and/or the oxygen free radicals formed during the oxidation. In our method we measure the degree of autoxidation by detection of adducts with cysteine formed in vivo. So far, we have found 5-S-cysteinyldopamine, -dopa and -dopac in several mammalian brains. We are, for the time being, involved in several studies where we analyze the adduct concentrations in the brain. They include two age studies, studies on brain tissue from patients with and without degeneration of dopaminergic areas and animal studies after different pharmacological manipulations. Initial data suggest that there is an increase in cysteinyl adduct levels with age, which may be explained by a weakened cellular defense system. The results from our age studies on humans and on guinea-pigs will be discussed at the symposium.

433.2

ALTERATIONS IN TYROSINE (TYR) AND TRYPTOPHAN (TRYP) CONCENTRATIONS IN RAT BRAIN AND PLASMA BY CLENBUTEROL (CLEN). D.J. Edwards and D.A. Sorisio*. Dept. Pharmacol.-Physiol. Univ. Pittsburgh Sch. Dent. Med., Pittsburgh, PA 15261.

Recent studies in our laboratory showed that the β_2-adrenoceptor agonist salbutamol decreases plasma TYR and raises brain TRYP concentrations (Life Sci. **42**: 853, 1988). We have now examined the effects of CLEN, a β_2-agonist which more readily penetrates the blood-brain barrier. Rats were injected i.p. with either 5 mg/kg CLEN or saline and decapitated 90 min later. In some experiments, the rats were pretreated with 15 mg/kg propranolol (PROP) or saline 20 min before CLEN or saline. TYR and TRYP were assayed by HPLC with electrochemical detection. CLEN lowered both TYR and TRYP in plasma and raised them in brain. Levels of these amino acids were either unchanged or decreased in heart, lungs, spleen and liver. These changes were only partially if at all reversed by PROP. A dose-response study revealed that the reductions in plasma TYR and the elevations in brain TRYP were equal over the range of 0.5-5 mg/kg of CLEN, but the effects of the lowest dose were completely antagonized by PROP. The effects of CLEN were not blocked by the serotonin (5-HT) antagonist, methysergide, ruling out an involvement of 5-HT receptors. Preliminary results suggest that the elevations in brain TYR and TRYP levels are due to stimulation of β_2-receptors, since the effects are blocked by the β_2-antagonist ICI 118,551 but not by the β_1-antagonist betaxolol. Supported by grant #MH28340.

433.3

THE EFFECT OF NIGRAL STIMULATION ON THE ACCUMULATION OF β-PHENYLETHYLAMINE (PE) IN THE RAT STRIATUM. A.V.Juorio* and I.A.Paterson (SPON: A.A.Boulton). Neuropsychiatric Res. Unit, Univ. of Saskatchewan, Saskatoon, Sask,Canada.

PE is a neurophysiologically active compound that occurs in small concentrations in the brain where is rapidly metabolized by type B monoamine oxidase (MAO). Earlier experiments have shown that unilateral electrolytic or 6-hydroxydopamine lesion of the rat substantia nigra (SN) produced decreases in PE levels in the ipsilateral striatum but no changes were observed after raphé nuclei lesion. The object of these experiments is to determine the extent of PE accumulation following stimulation of the SN. The effect of stimulation of the SN (20 Hz, 100 µA, 1 hour with bipolar concentric electrodes) on the striatal accumulation of PE in anaesthesized (urethane 1 g/kg, i.p.) was determined in deprenyl pretreated rats (2mg/kg, i.p., 3 hours). The determination of PE was performed by a mass spectrometric technique and DA, DOPAC and HVA by HPLC with electrochemical detection. The SN stimulation produced a small but significant reduction (to 78 % of controls) in the accumulation of striatal PE at a time when the concentrations of either DOPAC or HVA were increased by about 50 % of their control levels. It may be that in MAO inhibitor treated rats, SN stimulation increases PE utilization and removal from the brain, or the increased rate of DA synthesis decreases the availability of phenylalanine for decarboxylation and synthesis of PE. Supported by Saskatchewan Health and a Saskatchewan Health Research Board Fellowship (I.A.P.).

433.4

THE RELATIONSHIP IN MANIC DISORDER OF CSF AMINE METABOLITES TO CLINICAL CHARACTERISTICS AFTER LITHIUM TREATMENT. CL Bowden, E Seleshi*, S Contreras*, MA Javors*, JW Maas. Departments of Psychiatry, The University of Texas Health Science Center, and Audie L. Murphy Memorial Veteran's Hospital, San Antonio, TX 78284.

Despite the clinical importance of manic disorder, and the general efficacy of lithium in its treatment, relatively little information is available about biological characteristics of manic disorder, or the effects of lithium on biological parameters. We studied the CSF amine metabolites MHPG, 5-HIAA and HVA in manic and bipolar depressed patients both at baseline and following four weeks treatment with lithium carbonate. Patients were diagnosed by Research Diagnostic Criteria, utilizing the Schedule for Affective Disorders and Schizophrenia. In addition to data from 18 manic patients and 20 unipolar depressed patients, baseline data were obtained from 9 healthy control subjects, and 29 patients with unipolar depression. Amine metabolite analyses were by HPLC with electrochemical detection. At baseline, CSF MHPG, but not 5-HIAA or HVA, was higher in manic patients than in healthy controls (12.0 ± 9.04 vs. 6.66 ± 2.36 pmol/ml, $\bar{x} \pm$ S.D.). After treatment, 5HIAA was higher in manic patients than in bipolar depressed patients, and the increase in 5HIAA was greater in manic patients than in bipolar depressed patients. In manic patients at baseline there was no significant correlation between MHPG and 5-HIAA. After four weeks treatment the metabolites showed a correlation of 0.77, $p = .01$. Whereas the correlation between change in MHPG and 5-HIAA was not significant for all manic patients ($r = 0.30$), when only clinically recovered cases were analyzed, the correlation was significant ($r = 0.82$). Data will also be presented with respect to the change in amine metabolites with treatment and the association of change in animal metabolites with change in symptomatology.

433.5

ORIGINS OF NOREPINEPHRINE IN RAT CEREBROSPINAL FLUID. E. Mamalaki*, L. S. Brady, D. Goldstein*, and M. Herkenham (SPON: M. A. Smith). Unit on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, and Hypertension-Endocrine Branch, NHLBI, Bethesda, MD 20892

The cerebrospinal fluid (CSF) contains informational substances whose levels fluctuate with alterations in CNS activity. Sources of catecholamines in cerebrospinal fluid (CSF) are poorly understood. The locus coeruleus is the main source of norepinephrine fibers in brain, and the superior cervical ganglion provides dense noradrenergic innervation of the choroid plexus. CSF was drawn from the cisterna magna in rats 4 days before either bilateral superior cervical ganglionectomy or electrolytic destruction of the locus coeruleus and again 4-7 days after the operations. Samples (100-200 µl) of CSF were assayed for catechols by liquid chromatography with electrochemical detection after batch alumina extraction. Ganglionectomy (N=5) did not significantly affect CSF levels of catechols, whereas locus coeruleus lesions (N=17) decreased CSF norepinephrine ($p<0.0001$) but not dihydroxyphenylalanine (DOPA), dopamine, dihydroxyphenylacetic acid (DOPAC), or dihydroxyphenylglycol (DHPG). Epinephrine was below detection limits. The decrease in CSF norepinephrine was correlated significantly ($r=0.50$, $p<0.05$) with the extent of the locus coeruleus lesions; however, total bilateral destruction decreased CSF norepinephrine by only 50%. The data indicate that the locus coeruleus is a major--but not exclusive--source of CSF norepinephrine.

433.6

A COMPARATIVE STUDY OF THE LEVELS OF MONOAMINES IN SELECTED AREAS OF THE RAT CEREBRAL CORTEX N.Kabani*, R.W.Dykes, T.A.Reader. McGill University,Universite de Montreal,Montreal, PQ.

HPLC was used to measure the levels of monoamines (MA) in the hindlimb region of the somatosensory cortex (SS), primary visual cortex (VIS), and the anterior cingulate cortex (CING) of adult male Sprague-Dawley rats.

Large, correlated changes were observed in different months of the year. A significant positive correlation ($r=0.9$) was seen between changes in 5-HT and DA in the SS whereas in the CING, a significant positive correlation was seen for all 3 primary MAs. In the VIS there was a negative correlation between NE and 5-HT ($r=0.9$). A highly significant difference was observed in the MA levels of the CING when compared to SS and VIS for all compounds except 3MT and E. Significant differences between SS and VIS were seen for NE, DA, DOPAC and HVA. Differences between the sensory corticies and CING is consistent with the functionally distinct role of the former and a difference in the levels of MAs within SS and VIS, is suggestive of a functionally different role for MAs in these two sensory corticies.
(Supported by MRC of Canada)

433.7

EFFECT OF MORPHINE ON EPINEPHRINE CONCENTRATION IN RAT BRAIN. M. Ota*, I. N. Mefford* and M. Linnoila. LCS, NIAAA and LCS, NIMH, Bethesda, MD 20892

Acute and chronic morphine (MO) administration affects the function of catecholamine (CA) systems within the central nervous system (CNS). Most investigators have studied effects of MO on the noradrenergic and dopaminergic systems, while only a few reports concern effects of MO on CNS epinephrine (EPI) concentration. We quantified acute and chronic effects of MO on concentrations of EPI, norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) in hypothalamus (HT), medulla, locus coeruleus and frontal cortex of male Sprague-Dawley rats. HPLC with electrochemical detection was used for quantification of the analysis.

A single injection of MO sulfate (20mg/kg s.c.) reduced EPI and NE contents only in the HT, while DA, DOPAC and HVA concentrations were elevated in all areas (EPI, control 53.4±10.1, n=4, 2hrs post-injection 28.8±6.5ng/g tissue, n=4, $p<0.01$). All changes were antagonized by pretreatment with naloxone hydrochloride (NAL)(1mg/kg i.p.). This finding suggests that MO exerts its effect on metabolism through the mu opiate receptors. Repeated twice-daily MO injections for 14 days (M14) did not produce significant changes in EPI, NE or DA concentrations in any area examined 2hrs after the last MO injection. Coadministration of NAL (1mg/kg i.p.) with MO in the last injection (M14+N) induced significant depletion of EPI and NE in the HT compared to the MO only treated group (EPI, M14 51.7±8.8, n=7, M14+N 35.1±7.2ng/g tissue, n=6, $p<0.01$). These results suggest that acutely administered MO increases EPI release in the HT. Tolerance develops to this effect. Following chronic MO administration, a low dose of the opiate antagonist NAL produces an increase in EPI release.

433.8

CONTINUOUS AND INTERMITTENT LEVODOPA ADMINISTRATION DIFFERENTIALLY AFFECT BEHAVIORAL AND BIOCHEMICAL INDICES OF CENTRAL DOPAMINGERGIC ACTIVITY. T.M. Engber, J.L. Juncos*, Z. Susel*, R. Raisman*, F. Thibaut*, Y. Agid* and T.N. Chase. NINCDS, Bethesda, MD and INSERM U-289, Hopital de la Salpetriere, Paris, France.

Levodopa (LD) treatment schedules have been implicated in the pathogenesis of motor response fluctuations in Parkinson's disease. We compared continuous and intermittent LD replacement therapies in male rats with unilateral 6-hydroxydopamine lesions of the ascending dopaminergic pathways; rats were divided into 15 and 30 day treatment groups. In each, 3 subgroups of 7-9 rats were treated with either: a) continuous (cont.) saline (infused i.p. via Alzet osmotic pumps) + saline injections [all injections (inj.) given i.p., b.i.d.]; b) cont. LD (all LD doses=100 mg/kg/day) + saline inj.; or c) cont. saline + LD inj. Three days after the last treatment, contralateral rotations induced by apomorphine (0.05 or 0.5 mg/kg s.c.) were recorded. Animals were then sacrificed, and the striata dissected and analyzed for ipsilateral/contralateral D1 ([3H]SCH 23390) and D2 ([3H]spiperone) dopamine receptor binding, glutamic acid decarboxylase (GAD) and tyrosine hydroxylase (TH) enzyme activities. Only rats with 99% depletion of TH in the lesioned striatum were used. The LD injected rats exhibited a significant increase in the number of rotations during the first hour compared to the other treatment groups. GAD activity was significantly increased in the lesioned striata and in the LD inj. group compared to the other treatment groups. Rotations in the first hour correlated with the increase in GAD activity but not with alterations in D1 or D2 binding. This finding of a relationship between behavioral hypersensitivity and striatal GAD acitivity, but not with dopamine receptor binding, suggests that this behavioral hypersensitivity involves mechanisms downstream from striatal dopamine receptors.

433.9

BEHAVIORAL AND BIOCHEMICAL EFFECTS OF REPEATED APOMORPHINE TREATMENTS IN RATS. B. A. Mattingly1, J. K. Rowlett*1, J. Graff*1, M. T. Bardo2 1: Morehead State Univ. Morehead, KY 40351. 2: Dept. of Psych., Univ. of Kentucky, Lexington, KY 40506

Repeated treatments with the dopamine receptor agonist, apomorphine (APO), induces behavioral sensitization to the locomotor stimulating effects of APO. The purpose of the present study was to determine whether this behavioral sensitization effect is related to changes in dopamine receptors or metabolism. In two experiments, rats were injected SC with either apomorphine (5 mg/kg) or vehicle and tested for locomotor activity daily for 13 days. Following this chronic treatment, all rats were sacrificed, brains were removed, and striatal and mesolimbic tissue was dissected. In Exp. 1, a ^{3}H-spiroperidol receptor binding assay was performed. In Exp. 2, dopamine and DOPAC levels were measured using high pressure liquid chromatography with electrochemical detection. The results revealed no significant differences in receptor binding, dopamine levels, DOPAC levels, or DOPAC/DA ratios between the APO and vehicle pretreatment groups. These findings suggest that the development of behavioral sensitization to apomorphine is not the result of a drug-induced increase in dopamine receptors or metabolism.(This research was supported by grants from Morehead State Univ. and the KY EPSCoR committee).

433.10

DIFFERENTIAL DOPAMINE AGONIST EFFECTS ON THE INHIBITION OF ARYLSULFATASE-C. E.J. Martin* and T.J. Shickley (SPON: R.O. Warwick,Jr.), Dept. of Pharm/Tox, Philadelphia College of Pharmacy and Science, Phila., PA 19104.

We have previously shown that dopamine (DA) inhibits arylsulfatase-C (ARS-C) (Neurosci. Abstr. 13 (2),p1474, 1987). We also have shown this inhibition to be competitive (Neurosci. Abstra. 14, 1988). In the present study we examined the ability of the DA agonists apomorphine (APO), SK&F 82526 and LY-171555 to produce inhibition of ARS-C.

Partially purified ARS-C (Sigma, S-1629) was incubated in the presence of DA, APO, SK&F 82526 and LY-171555 using a modification of the spectrophotometric technic of Fowler and Rammler (Biochem. 3, p230, 1964).

The D1 agonist SK&F 82526 and the D2 agonist LY-171555 had no ability to block the desulfation of p-nitrophenyl sulfate at concentrations up to 1E-3M. The partial agonist APO produced inhibition similar to that of DA. These results suggest a structure activity relationship for the inhibition of ARS-C by DA and APO which is independent of the receptor-specific sites of the DA agonists SK&F 82526 and LY-171555.(Supported by USPHS Grant NS-26040 to T.J.S.)

433.11

ENZYME KINETICS OF DOPAMINE INHIBITION OF ARYLSULFATASE-C T.A. Cawley,Jr., E.J. Martin* and T.J. Shickley. Dept. Pharm/Tox, Philadelphia College of Pharmacy and Science, Phila. PA 19104

Arylsulfatases (EC 3.1.6.1) occur in nature in three distinct forms: A,B and C (ARS-C), all of which are found in brain. Little is known about the role of ARS-C in the CNS.

It has been previously reported that dopamine (DA) produces inhibition of ARS-C (Enyedy and Shickley. Soc. Neurosci. Abstr. 13,(2) p.1474, 1987). We have investigated the kinetics of this inhibition by DA on ARS-C.

Partially purified ARS-C (Sigma,S-1629) activity was assayed spectrophotometrically using p-nitrophenyl sulfate (p-NPS) as substrate by measuring enzymatically liberated p-nitrophenol (p-NP). Inhibition was analyzed by varying DA concentration in the presence of fixed concentrations of p-NPS.

Kinetic analysis of inhibition (Dixon Plot) revealed an apparent K_i for DA of approximately 600 uM, which is well below the DA concentration in limbic forebrain tissue (Anden et al. Acta Physiol. Scand. 67,p.306-312, 1966). This inhibition was found to fit a model for simple competitive inhibition. (Supported by USPHS Grant NS-26040 to T.J.S.)

433.12

AGE-DEPENDENT EFFECTS OF NIGROSTRIATAL LESIONS ON DOPAMINERGIC MODULATION OF ACETYLCHOLINE RELEASE. D. Jackson, S. Bernath, J.P. Bruno and M.J. Zigmond. Univ. of Pittsburgh, Pittsburgh, PA. 15260.

Adult rats are dependent upon dopamine (DA) for normal behavior: destruction of nigrostriatal bundle (NSB) results in severe behavioral deficits and, if recovery occurs, deficits can be reinstated with DA receptor antagonists. In contrast, NSB lesions produced in neonates cause no such deficits and DA antagonists have little behavioral effect. We have examined the role of residual DA in striatal function in rats lesioned during development with 6-hydroxydopamine (6-HDA). At adulthood, striatal slices were preincubated with [3H]choline, superfused with Krebs bicarbonate buffer, and exposed to electrical field stimulation (8 Hz, 1 min). The ability of sulpiride to stimulate tritium overflow was used as an index of DA inhibition of ACh release. DA in superfusates was quantified with HPLC. Although DA overflow was reduced by 6-HDA, fractional DA overflow was increased above control levels when slices were prepared from rats lesioned at 20 days of age or as adults; no such effect was seen when lesions were made at 3 or 15 days of age.Moreover, endogenous DA appeared to inhibit ACh overflow only in slices from rats lesioned at 20 days of age or greater. These results parallel our behavioral observations and suggest that if NSB injury is sustained at a young age, normal function may develop without the need for a dopaminergic influence. (Supported by NS19608)

433.13

EFFECTS OF UNILATERAL 6-HYDROXYDOPAMINE LESIONS OF SUBSTANTIA NIGRA ON DOPAMINERGIC TRANSMISSION IN RAT STRIATUM. W. Zhang*, S.J. Li*, H. Tilson, K. Nanry*, P. Hudson*, J.S. Hong, and M.K. Stachowiak.(SPON:R.H. Rech). Lab. Molecular and Integrative Neuroscience,NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709.

Unilateral injection of 6-HDA (8 ug) into substantia nigra produced increases in met-enkephalin (ME) content in the ipsilateral striatum. Increase in ME immunoreactivity occurred only in striata with greater than 90% dopamine (DA) depletions suggesting that the enhanced efficiency of DA transmission may compensate for the less extensive denervation. In rats with greater than 70% DA depletion, supersensitivity of striatal dynorphin neurons to apomorphine (1 mg/kg, i.p.) was observed. Presynaptic compensations were examined by measuring extracellular concentration of striatal DA using brain dialysis. Concentration of DA in dialysates remained unchanged in striata with 60-90% DA depletions. More extensive lesions were accompanied by only 60% reduction of DA release. Increased DA release was also observed in striatum contralateral to 6-HDA lesion and was accompanied by increased DA and HVA tissue content.

In conclusion, our results indicate that DA transmission in striatum is sustained following depletion of up to 90% of striatal DA. Such synaptic homeostasis may involve increased DA release, and following more extensive lesions, increased responsiveness of striatal neurons to DA.

433.14

REDUCTION OF DOPAMINERGIC INPUT INCREASES THE EXPRESSION OF THE ENKEPHALIN GENE. G.R. Christoph, B. Burkhart*, R. G. Krause II*, J. Angulo, M.E. Lewis and L.G. Davis. Medical Products Dept., E.I. Dupont de Nemours & Co., Inc., Wilmington, DE 19898.

We have investigated the effect of dopamine receptor blockade on striatal proenkephalin mRNA levels by Northern gel analysis and in situ hybridization. Chronic haloperidol treatment resulted in a 3.5-fold increase in striatal proenkephalin mRNA. This effect could be blocked with a co-administration of apomorphine. Changes in proenkephalin mRNA levels were uniform throughout the caudate-putamen as determined by in situ hybridization. Furthermore, unilateral 6-OHDA lesions of the substantia nigra, which reduced dopaminergic input to the striatum, result in a significant ipsilateral elevation of proenkephalin mRNA. This elevation can be reversed by transplanting embryonic ventral mesencephalic tissue, which contains dopaminergic perikarya, into the denervated striatum. The results imply that altering receptor-mediated neurotransmitter activity can lead to alterations in neuronal gene expression.

To determine if the increase in proenkephalin mRNA involved transcriptional regulation, heteronuclear RNA (hnRNA) was assayed with an intron containing probe. Our data indicate that proenkephalin mRNA and hnRNA are similarly affected by dopaminergic receptor blockade, suggesting that regulation is at least partly at the level of gene transcription. Experiments in progress are aimed at understanding the biochemical mechanisms in the transduction of information from the dopamine receptor to the nucleus that leads to the alteration of the proenkephalin mRNA levels.

434.1

D-2 AGONIST QUINPIROLE INDUCES INHIBITION AND EXCITATION OF FORWARD PROGRESSION. D. Eilam and H. Szechtman. Dept. Biomedical Sciences, McMaster Univ., Hamilton, Ontario, CANADA L8N 3Z5.

The effect of the D-2 agonist quinpirole (LY171555) on forward progression was measured in 60 rats injected with either saline, 0.03, 0.125, 0.5, 2, or 8 mg/kg of the drug. The total amount of forward progression was measured continuously during the two hour observation period. Results indicate that across both dose and time, quinpirole induces inhibition and then excitation of forward progression. Thus, the 0.03 mg/kg dose of quinpirole inhibited forward progression to nil in all rats, and doses higher than 0.125 mg/kg elevated it (compared to saline). At 0.125 mg/kg, locomotion was inhibited in some rats and elevated in others, suggesting that this dose is at the threshold of the switch from inhibition to excitation. Across time, a similar profile was evident. In the first 20 minutes after injection of 0.5 mg/kg or more of quinpirole, locomotion was inhibited (compared to saline animals) and elevated dramatically from 60-120 min of testing. This behavioral profile may parallel electrophysiological findings of disinhibition and inhibition of nucleus accumbens neurons by D2 receptor stimulation [Hu & Wang, (1988) Brain Res. 444:389]. (Supported by MRC. HS is a Research Associate of the Ontario Mental Health Foundation.)

434.2

COMBINED, BUT NOT SEPARATE, INJECTION OF D1 (SKF 38393) AND D2 (LY 171555) DOPAMINE AGONISTS INTO THE NUCLEUS ACCUMBENS INCREASES LOCOMOTOR ACTIVITY. T.J. Walsh D.F. Emerich and L.A. Taylor *. Rutgers University, Deparment of Psychology, New Brunswick, NJ 08903.

It is well established that the nucleus accumbens (NA) and its dopaminergic innervation from the ventral tegmental area (A10) are involved in the modulation of goal-directed motor behavior. The systemic and intraaccumbens administration of indirect and direct acting DA agonists such as amphetamine and apomorphine increase motor activity in a DA - dependent manner. Pharmacological and neurochemical evidence indicates that there is a duplicity of DA receptors which are designated D1 and D2 (Kebabian, J.W. Nature, 277, 93, 1979). The functional properties of these receptors in the control of motor behavior, however, have not been well characterized.

The present study demonstrated that bilateral injection of either D1 (10 or 20 ug SKF 38393) or D2 (0.5, 1.0, or 2.0 ug LY 171555) agonists into the NA of male Sprague dawley rats produced no significant increases in motor activity compared to saline injected controls. However, combined administration of the D1 and D2 agonists in a "cocktail" did significantly increase motor activity in a dose-related fashion. Combined administration of 15 ug of SKF 38393 and 1.25 ug LY 171555, or 10ug of SKF 38393 and 0.5 ug LY 171555, increased motor activity 119 % - 296% over control values for up to 90 minutes following injection.

Our results are consistent with recent electrophysiological studies that have shown that concurrent D1 and D2 receptor stimulation in the NA may be necessary to initiate and direct locomotor behavior. (Clark D. et. al., Synapse, 1, 347, 1987). These results indicate a coordinated involvement of DA receptor subtypes in the modulation of behavior.

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434.3

EFFECTS OF D1 AND D2 RECEPTOR STIMULATION ON AROUSAL, ATTENTION AND INTENTION TO MOVE. A. R. Braun* and T. N. Chase (SPON: J. Walters). Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20892

Dopamine appears to play a significant role in the mechanisms of arousal, selective and sustained attention, and intention to move. In order to characterize the relative roles of D1 and D2 receptors in these processes we administered the selective D1 and D2 agonists SKF38393 and LY171555 alone and in combination to male rats and evaluated responses to several categories of environmental stimuli. Both D1 and D2 receptor agonists produced dose dependent increases in arousal and in orientation to novel objects and discrete moving stimuli. Selective D2 receptor stimulation resulted in decreases in response to noxious stimuli while the D1 agonist had no significant effect. The D2 agonist produced increases in sustained exploration of a novel object and pursuit of a moving stimulus, while D1 receptor stimulation produced decreases in these behaviors. The D2 agonist produced increases in the time animals initially spent in the center of an open field, but had no significant effects upon other open field behaviors; D1 receptor stimulation did not affect time spent in the center of the field but produced dose dependent decreases in investigatory behavior and increased disorganization of open field locomotor patterns. When the agonists were administered together in varying doses, the same overall patterns persisted; concurrent D1/D2 receptor stimulation had pronounced interactive effects upon sustained attention and exploratory behavior. There was in addition an apparent functional distinction between high and low doses of the D1 agonist. When endogenous dopamine was depleted by AMPT pretreatment, D1 receptor stimulation continuted to produce an increase in orientation but did not affect other parameters. D2 receptor stimulation, on the other hand, produced dose dependent decreases in orientation, exploration, and locomotion in an open field. These data provide evidence of distinct and generally opposing roles of D1 and D2 receptor stimulation upon responses to environmental stimuli and evidence of D1/D2 receptor interaction in the generation of such responses. The data also suggest that reduction in D1 receptor tone produces sensorimotor responses following D2 receptor stimulation which are functionally the opposite of those seen in otherwise intact animals following D2 agonist administration.

434.4

CHARACTERIZATION OF RABBIT POLYCLONAL ANTI-IDIOTYPIC ANTIBODY AGAINST CONJUGATED ANTI-DOPAMINE. N. Mons*, M. Geffard, C. Messier (1), O. Mrabet (1) and C. Destrade (1). Lab. Neuroimmunology, IBCN-CNRS, 33077 BORDEAUX and (1) Lab. Psychophysiologie, UA CNRS 399, 33405 TALENCE FRANCE.

This study examined the raising of rabbit polyclonal anti-idiotypic antiserum (Ab2) that recognize dopamine (DA) receptors. Our approach to obtain this anti-idiotypic antibody (Ab) has consisted : (i) in developing polyclonal and monoclonal anti-DA conjugated antibodies which had high affinity for DA-G-lysine, (ii) in raising antibodies (Ab2) against these idiotypic antisera (Ab1). Rabbit polyclonal Ab2 was obtained after alternate immunizations with either mouse Ab or purified rabbit IgG with complete Freund's adjuvant. Then, the Ab2 were affinity chromatographed to remove anti-isotypic and anti-allotypic antibodies. The specificity of the crude anti-idiotypic was tested (1) in ELISA for their capacity to bind poly- and monoclonal idiotypic sites, (2) for its ability to inhibit 3H DA binding to DA-receptors: the 3H DA was displaced at 1/10000 dilution, (3) for its immunohistochemical visualization. Male rats were fixed with paraformaldehyde 4%-picric acid 0.2%. Fifty m sections were subjected to PAP method, using a 1/1000 dilution of Ab2. Immunoreactive product was observed in striatum, septum and substantia nigra and was abolished after preadsorption with monoclonal or polyclonal Ab1, (4) Unilateral intra-accumbens injection of the anti-idiotype in amphetamine pre-treated mice produced ipsilateral circling.

434.5

ROTATIONAL BEHAVIOR PRODUCED BY INTRA-ACCUMBENS MICROINJECTION OF CONJUGATED DOPAMINE ANTI-IDIOTYPIC ANTIBODIES. O. Mrabet*, C. Messier, N. Mons (1), M. Geffard (1) and C. Destrade. (SPON: European Brain and Behavior Society). Lab. Psychophysiologie, UA CNRS 339, TALENCE FRANCE and (1) Lab. Neuroimmunologie, IBCN-CNRS, BORDEAUX FRANCE.

Polyclonal dopamine (DA) anti-idiotype antibodies were raised in immunized rabbits with either purified polyclonal immunoglobulins or a monoclonal anti-conjugated DA antibody (Chagnaud et al., J.of Neurochem., 49, 487-494, 1987). Anti-idiotype antibody affinity and specificity were evaluated. In the present experiments, we tested the ability of the DA anti-idiotypic (AI) antibodies to change behavior through its action on a brain area rich in DA receptors. We microinjected the DA AI antibodies unilaterally into the nucleus accumbens of 2 mg/kg amphetamine pre- treated BALB/c mice. One group was injected with either 0.5, 1.5 or 3.5 µl of DA AI; a second group was injected with either 0.5, 1.5 or 3.5 µl of immunoglobulins from non-immune mice (IgG); one last group was injected with 1.5 µl of DA AI followed 45 min later by an intra-accumbens injection of apomorphine (40 ng in 2 µl). The injection of DA AI produced a locomotion bias which resulted in ipsilateral turning. No locomotion bias was observed in the mice injected with either of the IgG doses. Injection of 1.5 µl of DA AI produced ipsilateral turning which was abolished and then reversed (contralateral turning) by the intra-accumbens apomorphine injection.

434.6

ELECTROPHYSIOLOGICAL AND BEHAVIORAL STUDIES OF AMINOTETRALINS (AT) IN RAT BRAIN DOPAMINE SYSTEMS. R.J.Brooderson and F.J. White. Depts. of Pharmacol. & Psychiat. Wayne St. Univ. Sch. Med., & Neuropsychopharm. Lab., Lafayette Clinic, Detroit, MI 48207

The ATs constitute a novel class of dopamine (DA) agonist with structures similar to DA itself. These compounds have been reported to stimulate both D1 and D2 DA receptors. Given recent findings indicating the necessity of stimulating both DA receptors for the functional expression of postsynaptic D2 responses, such mixed agonists may potentially be of therepeutic utility.

We have begun to characterize the electrophysiological effects of several AT DA agonists within the mesoaccumbens DA pathway using extracellular single-unit recording techniques in chloral-hydrate anesthetized rats. Recordings from A10 DA cells indicate that the rank order of potencies at somatodendritic DA autoreceptors is: (±)-5-OHDPAT (ED_{50} 2.3 µg/kg) = (±)-7-OHDPAT (ED_{50} 2.6 µg/kg) > (±)-Dimethyl-5,6-dihydroxy-ADTN (ED_{50} 14 µg/kg) ≥ (±)-Dimethyl-6,7-dihyroxyADTN (TL-99; ED_{50} 18 µg/kg). Thus, the monohydroxylated ATs appear more potent than their dihydroxylated counterparts. Preliminary behavioral results indicate that both 5- and 7-OHDPAT (0.5mg/kg, s.c.) can reverse akinesia produced by reserpine+αMPT and induce stereotyped behaviors in rats, effects which reqiure stimulation of both D1 and D2 receptors. Thus, these ATs are potent agonists at somatodendritic DA autoreceptors and also exert both D1 and D2 agonist effects postsynaptically.

434.7

COMPLEX DOPAMINE (DA) AGONIST/ANTAGONIST EFFECTS ON SUBSTANTIA NIGRA PARS RETICULATA (SNpr) NEURONS. L.Martin* and B.L. Waszczak. Pharmacol. Sect., Northeastern Univ., Boston, MA.

Previous studies suggested that the ability of DA to increase firing and to lessen responses of SNpr neurons to GABA could be mediated by D-1 and D-2 receptors, respectively. Extracellular recordings were carried out in rats to examine the selectivity of these actions. Surprisingly, iontophoretic application (2 and 5 nA) of neither (-)sulpiride (SUL; 0.2M) nor zetidoline (ZET; 0.2M), both selective D-2 antagonists, could prevent the modulation of GABA effects by the D-2 agonist LY 171555 (LY; 0.05M, 10 nA). In fact, both drugs caused current-related agonist-like attenuations of GABA's actions, and acted additively or synergistically with LY to further depress responses to GABA (n=7-8 cells). A similar pattern was also observed for (+)SUL (0.2M; n=6) at 2 and 5 nA (enantiomeric purity was confirmed by polarimetry). At the same currents, ZET and both (-) and (+)SUL could, however, block slowing of SN DA cell firing by LY.

In related studies, analogs of cAMP (0.2M; 5-30 nA) and forskolin (FOR; 1mM; 5-15 nA) were compared with the D-1 agonist SKF 38393 (SKF). While applied FOR (n=5) and dibutyryl cAMP (n=10) acted like SKF to increase SNpr firing without lessening GABA effects, 8Br-cAMP (n=9) caused both current-related increases in firing and modulation of GABA responses, similar to D-2 agonist actions. These results confound efforts to delineate D-1 and D-2 effects on SNpr cells, and advise caution in use of these drugs by iontophoresis methods to decipher such mechanisms. Support: NS23541

434.8

EFFECTS OF SELECTIVE D1 AND D2 RECEPTOR AGONISTS ON THE ACTIVITY OF PREFRONTAL CORTEX CELLS IN FISCHER-344 RATS. A. Gratton, P. Bickford-Weimer, K.D. Parfitt. Douglas Hosp. Res. Ctr, McGill Univ., Montreal, Canada, H4H 1R3 and Dept. Pharmacol., Univ. Colorado Hlth Sci. Ctr, Denver, CO 80218.

The effects of the selective dopamine (DA) D1 and D2 receptor agonists, SKF38393 and N0437 respectively, on the firing rate of medial prefrontal cortex (MPFC) cells were studied in young (3-5 months old) Fischer-344 rats. Multibarrel glass micropipettes, filled with 1 mM SKF38393 and N0437, were lowered into the anteromedial cortical terminal field of the mesocortical DA system in urethane anesthetized animals. The drug solutions were locally applied by pressure ejection. Both drugs produced dose-dependent and reversible reductions in firing rates. However, the D2 agonist was approximately 10 times more potent than the D1 agonist in suppressing firing rate. The ED50 values for N0437 and SKF38393 were 9.9 and 118.3 psi-sec respectively. Moreover, even at the highest doses, SKF38393 rarely produced complete cessation of firing in MPFC cells. Finally no evidence of synergism was observed when the two drugs were simultaneously applied; the effects of one drug were not potentiated by the concurrent application of the other. The present data suggest that D2 agonists are far more potent than D1 agonists in MPFC.

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434.9

THE D-1 SELECTIVE AGONIST SKF 38393 CAN ACTIVATE STRIATAL NEURONS IN 6-HYDROXYDOPAMINE LESIONED RATS. B.G. Weick and J.R. Walters. NINCDS, Bethesda, MD 20892.

In rats with unilateral 6-hydroxydopamine (6-OHDA)-induced lesion of the nigrostriatal dopamine (DA) pathway, some evidence suggests DA agonists may activate an inhibitory striatal input to the substantia nigra pars reticulata (SNpr). Behavioral studies with these rats have attributed contralateral turning induced by DA agonists to increased GABA-mediated inhibition of SNpr cells. It has also been shown that i.v. apomorphine and the D-1 agonist SKF 38393 inhibit SNpr single unit activity and markedly increase glucose utilization in these rats. We have utilized extracellular single unit recording techniques in locally anesthetized, gallamine immobilized, artificially respired rats 6 -8 weeks after 6-OHDA nigrostriatal lesion to examine whether i.v. SKF 38393 administration does activate striatal output cells as these observations suggest. Firing rates of 73% of striatal cells with spontaneous activity between 1 and 8 spikes/sec were significantly elevated by i.v. SKF 38393. Increases averaged 2.1±0.8 spikes/sec at 1 mg/kg (n=11), 3.1±1.0 spikes/sec at 3.4 mg/kg (n=9) and 5.2±1.7 spikes/sec at 10 mg/kg (n=7). Increased firing was reversed by SCH 23390. In addition, 3 of 5 quiescent neurons antidromically activated from the nigra began to fire after 3.4 mg/kg SKF 38393. Two neurons with spontaneous firing rates greater than 8 spikes/sec were inhibited by SKF 38393. This study supports the idea that a population of striatonigral inhibitory neurons are stimulated by systemic administration of a D-1 agonist in rats after 6-OHDA lesions.

434.10

D1 DOPAMINE RECEPTOR STIMULATION ENABLES THE POSTSYNAPTIC BUT NOT AUTORECEPTOR EFFECTS OF D2 DOPAMINE AGONISTS. S.R. Wachtel, M.P. Galloway, & F.J. White. Depts. Pharmacol. & Psychiat., Wayne State Univ. Sch. of Med., & NPPL & NPRU, Lafayette Clinic, Detroit, MI 48207

Recent electrophysiological evidence suggests that D1 dopamine (DA) receptor stimulation enables the inhibitory effects of postsynaptic D2 receptor stimulation in the rat nucleus accumbens (NAc). In the present studies we have elaborated our previous findings in the NAc and extended them to the caudate nucleus (Cd). Depletion of DA via AMPT pretreatment attenuated the inhinbitory effects of the D2 DA agonist quinpirole (QUIN), but not those of the mixed D1/D2 agonist apomorphine. The inhibition by QUIN was restored by co-iontophoresis with the D1 agonist SKF 38393 (SKF). In contrast to the enabling role of D1 stimulation on postsynaptic D2 DA receptor-mediated inhibition, removal of D1 receptor stimulation by DA depletion failed to alter the inhibition by QUIN on A9 and A10 DA cells. Moreover, co-application of either SKF or the D1 antagonist SCH 23390 (SCH) failed to alter the inhibitory effects of QUIN on A9 and A10 DA firing. Synthesis-modulating DA autoreceptors were also unaffected by manipulations of D1 receptor activation. The reversal of the GBL-induced increase in striatal and NAc DOPA accumulation by QUIN was unaffected by SKF or SCH co-administration. Thus, neither the impulse-regulating nor the synthesis-modulating D2 autoreceptor was functionally enabled by D1 stimulation, further supporting the lack of a D1 DA autoreceptor.

434.11

NEUROPHYSIOLOGICAL EFFECTS OF (+)-UH 232 and (+)-AJ 76, DOPAMINE AUTORECEPTOR ANTAGONISTS, ON DOPAMINE PRE- AND POSTSYNAPTIC RECEPTORS. D.A. Bergstrom, M. Beninato and J.R. Walters. NINCDS, Bethesda, MD 20892.

(+)-UH 232 and (+)-AJ 76 exert behavioral and biochemical effects which suggest that they can act preferentially at dopamine (DA) autoreceptors as antagonists (Svensson et al., 1986). Extracellular single unit recording techniques were used to evaluate the abilities of these drugs to interact with nigra DA autoreceptors and postsynaptic DA receptors in the basal ganglia by comparing their effects on the activity of nigra pars compacta DA neurons and globus pallidus (GP) neurons, respectively, in locally anesthetized, gallamine immobilized, artificially respired rats. At 13 μmol/kg i.v., both drugs stimulated DA cell firing rates by 28% (n=14) and increased the ED_{50} for apomorphine (APO)-induced inhibition of DA cell activity by 40-70 fold (control ED_{50}: 10±2 μg/kg, n=9). Increases in GP neuronal activity induced by 0.3 mg/kg APO were effectively blocked by pretreatment or antagonized with 13 μmol/kg UH 232 (n=4) or AJ 76 (n=7). A 10-fold lower dose of (+)-UH 232 (1.3 μmol/kg) also stimulated DA cell activity by 27±6% (n=7), increased APO's ED_{50} on DA cells to 163±36 μg/kg (n=6) and effectively reversed APO-induced increases in GP cell activity (n=5). The same 1.3 μmol/kg i.v. dose of (+)-AJ 76 stimulated DA cell activity by 16±3% (n=7), increased APO's ED_{50} on DA cells to 24±5 μg/kg (n=6) and shifted the dose-response curve of APO's effects on GP neurons (n=16) to the right. With this experimental protocol, (+)-UH 232 and (+)-AJ 76 demonstrated antagonistic effects both at DA autoreceptors and at postsynaptic receptors.

434.12

PARTIAL RECEPTOR INACTIVATION SHOWS LOW EFFICACY OF S(+)NPA. B.L. Waszczak and R.F. Cox (SPON: J.L. Neumeyer). Pharmacol. Sect., Northeastern Univ., Boston, MA 02115.

Previous studies showed that S(+)NPA had dopamine (DA) agonist potency 300-fold lower than R(-)NPA in slowing firing of nigral (SN) DA neurons. Antagonist effects were also evident since pretreatment with 40 ug/kg S(+)NPA caused a significant rightward shift of the R(-)NPA dose-response curve (drc). Such actions suggested low intrinsic efficacy for S(+)NPA. To test this, we conducted dose-response studies for slowing of rat SN DA cell firing by i.v. R(-) and S(+)NPA after partial irreversible inactivation of DA receptors with 6mg/kg EEDQ (in ethanol). EEDQ pretreatment caused a significant parallel rightward shift of the R(-)-NPA drc but no loss of maximal response relative to ethanol control rats. The same dose of EEDQ reduced the maximal response to S(+)NPA by 22% with a small shift on the dose axis. Furchgott analysis gave a steep, hyperbolic occupancy response (O-R) relationship for R(-)NPA with 50% and 100% responses at 3.5% and 28% receptor occupancies, respectively. Thus, a 72% receptor reserve exists for R(-)NPA in in this model. The O-R plot for S(+)NPA was more shallow and linear (r=0.998). Half maximal effect occurred at 65.6% occupancy; maximal response (96% inhibition) was attained at 94% occupancy. Hence, few spare receptors are present for S(+)NPA. Since a ratio of fractional occupancies at a given response is a measure of relative efficacy, at 50% response the efficacy of S(+) relative to R(-)NPA is 0.05, confirming that S(+)NPA has low efficacy. Support: NS23541

435.1

REGIONAL DIFFERENCES IN THE BINDING OF PIRENZEPINE AND AF-DX 116 TO RAT BRAIN: COMPARISON WITH MINIMUM ENERGY CONFORMATIONS. W. Hoss, B.R. Ellerbrock*, D.A. Smith* and W.S. Messer, Jr. Dept. of Medicinal and Biological Chemistry, College of Pharmacy and Dept. of Chemistry, Univ. of Toledo, 2801 W. Bancroft St. Toledo, OH 43606

The binding of selective muscarinic receptor antagonists to regions of rat brain was examined through autoradiographic techniques. Pirenzepine and AF-DX 116 were chosen based on their selectivity for M_1 and M_2 muscarinic receptors respectively, and similarities in chemical structure. Pirenzepine displayed a higher potency than AF-DX 116 for the inhibition of [^{3}H]-l-quinuclidinyl benzilate binding to rat brain sections. Analyses of binding to brain sections revealed heterogeneous binding profiles for both antagonists, suggesting the presence of multiple receptor sites.

Quantitative autoradiographic techniques were utilized in regional analyses of pirenzepine and AF-DX 116 binding. Pirenzepine displayed the highest affinity for hippocampal, striatal and amygdaloid muscarinic receptors (IC_{50}'s < 0.4 μM), with a slightly lower affinity for cortical receptors (IC_{50}'s between 0.4 and 0.8 μM). Pirenzepine displayed the lowest affinity for thalamic and brainstem regions with IC_{50}'s generally > 1.0 μM. In contrast, AF-DX 116 bound with higher affinity to muscarinic receptors in brainstem, cerebellar and hypothalamic nuclei (IC_{50}'s < 0.5 μM) than to receptors in thalamic nuclei (IC_{50}'s between 0.5 and 2.0 μM). Binding sites with the lowest affinity for AF-DX 116 were found in cortical, striatal and hippocampal regions (IC_{50}'s > than 2.0 μM). The binding profiles of the two selective muscarinic antagonists reveal the complexity and diversity of muscarinic receptor subtypes throughout the brain. The data provide a basis for identifying muscarinic receptor subtypes with selective ligands.

Minimum energy conformations of pirenzepine and AF-DX 116 were calculated using the program MacroModel (version 1.5). Pirenzepine displayed three energy minima, differing in the relative position of the piperazine ring with respect to the tricyclic system. In contrast, the diethylaminomethyl substituent on the piperidine ring conferred a much larger set of minimum energy conformations on AF-DX 116. It is suggested that the greater conformational flexibility of AF-DX 116 allows it to achieve a conformation inaccessible to pirenzepine that can bind to M_2 receptors.

Supported by NS 23929.

435.2

AGONIST-RECEPTOR ACTIVE CONFORMATIONS FOR CENTRAL M1 AND M2 MUSCARINIC RECEPTOR-EFFECTOR SYSTEMS. M. McKINNEY, D. ANDERSON, L. VELLA-ROUNTREE*. Neuroscience Research Division, Pharmaceutical Discovery, Dept. 47W, Abbott Laboratories, Abbott Park, IL 60064

The coupling to biochemical effector systems of central M_1 receptors (cortical phosphoinositide metabolism) and M_2 receptors (cortical and striatal cAMP inhibition) was studied in metabolically-prelabeled, mechanically-dissociated cellular preparations of the adult rat brain. Cortical [^{3}H]IP1 formation was blocked by pirenzepine with high affinity (K_i=10 nM) while this antagonist blocked cortical and striatal forskolin-elevated [^{3}H]cAMP formation with K_i values of 354 nM and 325 nM, respectively, indicating coupling of the latter response to M_2 receptors. Propylbenzilylcholine mustard (IC_{50}=6 nM) was employed to partially occlude muscarinic receptors in this preparation and the equilibrium binding constants for carbachol in mediating M_1 and M_2 responses were determined. Carbachol was bound to the M_1 receptor with a K_d value identical to its EC_{50} (104 μM), while this agonist mediated the M_2 responses in cortex and striatum with K_d values of 15 μM and 5μM, respectively. These findings indicate that the central M_1 receptor is activated by the agonist binding in a low-affinity agonist-receptor conformation, while the central M_2 receptor is activated by the agonist in a high-affinity active conformation.

435.3

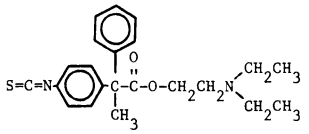
ASSESSMENT OF MUSCARINIC ANTAGONIST SELECTIVITIES: RESPONSE ASSAYS IN CULTURED CELLS COMPARED WITH BRAIN M1 AND M2 BINDING POTENCIES. D.J. Anderson, L. Vella-Rountree*, D. Barnes*, and M. McKinney. Neuroscience Research Division, Pharmaceutical Discovery, Dept. 47W, Abbott Laboratories, Abbott Park, IL 60064.

M1 and M2 muscarinic receptors mediating separate biochemical responses are found in mouse neuroblastoma clone N1E-115. The M1 receptor elevates cGMP, while the M2 receptor inhibits PGE1-elevated cAMP formation. We used these receptor-effector systems to assess selectivities of muscarinic antagonists. An "equipotent molar ratio" (EPMR=IC_{50ant}/IC_{50atr}) was calculated for each of twelve antagonists relative to atropine, to compare their selectivity for blockade of carbachol-mediated M1 and M2 responses. Pirenzepine was 30-fold M1-selective, in agreement with selectivity found with Schild analysis (McKinney et al., Mol. Pharmacol. 27:223, 1985). Several other antagonists were M1 selective: trihexyphenidyl (4-fold), QNX (8-fold), and benzhexol (3-fold). Four antagonists were M2-selective in this comparison: pancuronium (7-fold), secoverine (7-fold), AF-DX 116 (4-fold), and 4-DAMP (3-fold). Central M1 and M2 receptor binding potencies were measured by blockade of [^{3}H]pirenzepine binding in the rat cortex and by blockade of [^{3}H]QNB binding in the medulla-pons, respectively. Interesting similarities and differences arose when functional selectivities were compared to those determined by binding potencies.

435.4

APROPHIT: A POTENTIAL IRREVERSIBLE ANTAGONIST FOR MUSCARINIC RECEPTORS. A.H. Newman, H. Leader, J. Covington, M. Oleshansky, P.K.Chiang. Walter Reed Army Institute of Research, Washington, DC 20307-5100.

The development of selective irreversible ligands has proven to be an invaluable technique for the characterization, isolation and purification of many receptor systems. We now report the preparation and preliminary evaluation of an isothiocyanato-derivative of aprophen (aprophit, 1) as a potential irreversible antagonist of muscarinic receptors. Aprophit inhibited the acetylcholine-stimulated release of catecholamines from isolated, perfused guinea pig adrenal glands in a dose-dependent manner. This inhibition was not reversed by perfusing the tissue with Locke's solution and was not due to a non-selective alkylation by the isothiocyanate function. This preliminary data suggests that aprophit may be binding to muscarinic receptors in an irreversible manner.



1

435.5

CHARACTERIZATION AND AUTORADIOGRAPHIC DISTRIBUTION OF BINDING SITES FOR [^{3}H]AF-DX116, A PUTATIVE M_2MUSCARINIC RECEPTOR PROBE. W. Regenold*, D. Araujo and R. Quirion (SPON: N.P.V. Nair) Douglas Hospital Res. Ctr., Dept. of Psychiatry, McGill Univ., Verdun, Quebec, Canada H4H 1R3.

The use of selective muscarinic antagonists in radioligand binding studies has greatly enhanced our knowledge of muscarinic receptor heterogeneity. We extend these studies through the characterization and autoradiographic depiction of [^{3}H]AF-DX116 binding sites in the rat CNS. Incubations for all experiments were in Krebs buffer at 4°C for 1 hr. Saturation experiments demonstrate binding to an apparently single class (mean n_H = 0.86) of high affinity (mean K_d = 2.8 nM) sites. Competitive inhibition experiments with 5nM [^{3}H]AF-DX116 indicate competition for a muscarinic(K_i for atropine = 1.25 nM; K_i for nicotinic drugs > 100,000 nM); non-pirenzepine/M_1 (K_i for pirenzepine = 101 nM); saturable (K_i for AF-DX116 = 0.91 nM) site. In vitro autoradiography with 10 nM [^{3}H]AF-DX116 shows an M_2 receptor-like distribution with the highest site densities seen in thalamic and brainstem nuclei. In the hippocampus, binding sites are rather diffusely distributed . In sum, [^{3}H]AF-DX116 is a useful M_2 muscarinic receptor probe. The full chemical name of AF-DX116 is 11[[2-[(diethyl-amino)-methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]-benzodiazepine-6-one.

Supported by the Medical Research Council of Canada.

435.6

BINDING OF A PUTATIVE M2 SELECTIVE MUSCARINIC ANTAGONIST, [3H]AF-DX 116, TO MUSCARINIC ACETYLCHOLINE RECEPTORS IS DECREASED IN THE AGED RAT BRAIN. M. Watson, X. Ming* and B. Fucigna*. Dept. of Pharmacology, University of Medicine and Dentistry of New Jersey- New Jersey Medical School, Newark, N.J. 07103-2757.

Data obtained by the use of selective antagonists such as pirenzepine (PZ) and AF-DX 116 (11-2-[[2-[(diethyl-amino) methyl]-1- piperidinyl] acetyl]-5,11-dihydro-6H-pyrido (2,3-b) (1,4) benzodiazepine-6-one) in binding and functional assays has lead to the subclassification of muscarinic acetylcholine receptors (mAChR) into subtypes (TIPS Suppl. II: 46, 1986). A senescent rat model of aging was employed to assess any age-related alterations in M2 mAChR characteristics in numerous regions of the central nervous system (CNS). Binding assays for [^{3}H]AF-DX 116, a putative M2 selective ligand, were conducted as previously described. Briefly, membrane homogenates were incubated at 25°C for 60 min. Significant reductions in binding capacity (over 20 percent) were noted in many CNS areas when 18 month old rats were compared to 3 month old controls. The cerebral cortex showed the most substantial reduction. These data directly illustrate that there is a marked and widespread age-related reduction in M2 mAChR binding in the aged rat brain. One may speculate that there is a link between this decrease and the diminished cognitive capabilities which have previously been reported to accompany the aging process. Supported in part by FUMDNJ, a BRSG from NJMS, and MH-43024.

435.7

DEVELOPMENT OF M_2 MUSCARINIC RECEPTORS IN FETAL AND NEONATAL MOUSE BRAIN AND HEART. J-X. Wang*,W. Wang*, H.I. Yamamura and W.R. Roeske Depts. of Pharmacology and Internal Medicine, Univ. of Arizona, Col. of Med. Tucson, AZ 85724.

Development of M_2 muscarinic receptor (mAChRs) has been studied in the fetal, neonatal and adult CD-1 mouse brain and heart. The total mAChRs were determined using [^{3}H](-) QNB and the M_2 receptors determined using the selective ligand [^{3}H]AF-DX 116. The total mAChRs and the M_2 receptors in both tissues reached the adult level after 42 postnatal days and half maximal value at about 14-21 postnatal days. The concentrations of total brain mAChR and the M_2 receptors, calculated based on the protein or tissue contents, increased continuously from fetal to postnatal periods. In contrast, the heart mAChR concentrations, based on either protein or tissue contents, reached the adult level as early as at the birth. The concentration of heart mAChRs kept increasing after birth with a peak level at 14 postnatal days and droped down to a lower level in the adult, suggesting a preferential development of mAChRs during early postnatal period. The percentage of M_2 receptors vs total mAChRs was constant (19-26%) in the brain while it varied (45-70%) in the heart during the development. In conclusion, the murine neuronal and cardiac M_2 receptors showed differential patterns of development.

435.8

EFFECT OF METHOCTRAMINE ON MUSCARINIC RECEPTORS IN MURINE NEUROBLASTOMA CELLS. A.D. Fryer*, N.H. Lee, E.E. El-Fakahany (SPON: N.Khazan). Dept. of Pharmacology and Toxicology, Univ. of Maryland School of Pharmacy, Baltimore, MD, 21201.

Methoctramine (Met) is an antagonist with greatest affinity for cardiac M_2 muscarinic receptors which also discriminates between muscarinic receptors in the cortex (M_1) and glands (glandular M_2), (E.J.P.; 1988, 145,61). Its antimuscarinic effects were examined using N1E-115 mouse neuroblastoma cells (NB cells). NB cells have 2 muscarinic receptor subtypes, M_1 receptors which mediate phosphoinositide (PI) turnover, and glandular M_2 receptors which inhibit cyclic AMP (cAMP) formation. Calculations were made under the assumption that Met is a competitive antagonist, since at the doses used it did not alter the rate of dissociation of bound [^{3}H]N-methyl scopolamine (NMS) from the receptors. Met displaced NMS (0.2 nM) binding from only one site in NB cells (K_D of 120 ± 11 nM, n_H close to 1.0, data showed best fit to one site using LIGAND). Met was also an equipotent antagonist for PI (stimulated by 1.0 mM carbamylcholine (CBC)) and cAMP responses (measured using 25 μM forskolin in the absence and presence of 0.1 mM CBC). The K_D values were 132 ± 19 and 155 ± 25 nM respectively, and the n_H were close to 1.0. Thus, in NB N1E-115 cells, Met does not show the obvious selectivity for muscarinic receptor subtypes reported using other tissues. (Supported in part by NIH grants 1F32HL07691-01 CLN2, NS-24158, AG-07118, AG-00344.)

435.9

SELECTIVE ANTAGONISTS REVEAL HETEROGENEITY OF RAT BRAIN MUSCARINIC RECEPTORS THAT ACTIVATE INOSITOL-PHOSPHATES METABOLISM. C. Forray, C.L. Amrhein*, O.N. Kim* and E.E. El-Fakahany. Dept. of Pharmacology and Toxicology, Univ. of Maryland School of Pharmacy, Baltimore, MD 21201.

The selectivity of muscarinic antagonists was studied in functional as well as binding studies in dissociated cell preparations from rat brain cortex. When carbachol (CBC) stimulation of [^{3}H]inositol-phosphates accumulation was assayed with increasing doses of pirenzepine (PIR) it revealed the presence of two components with an IC_{50} ratio of 1000, and 50 to 70% as high affinity sites. Competition of [^{3}H]NMS by PIR in the same preparation also showed 62% high-affinity sites with an affinities ratio of 27. Methoctramine (MET) Schild plot estimated a pA_2 of 7.03 for the CBC response at low concentrations. However in the μM range MET induced non-competitive effects. In competition binding experiments MET showed a pKi of 7.1 for 93% of the sites and 4.9 for a low-affinity site. The PIR low-affinity component of CBC response assayed in the presence of 100 nM PIR showed the following rank order of potencies: 4-DAMP> HHSiD> PIR> AF-DX 116. Our data suggest that muscarinic receptors that stimulate inositol-phosphate metabolism in rat brain cortex are of two different subtypes. Evidence obtained so far points towards the involvement of "glandular M_2" in addition to the M_1 subtype in this response. (Supported in part by NIH grants NS-24158, AG-07118 and AG-0034).

435.10

BINDING OF BM-5 (N-METHYL-N-[1-METHYL-4-PYRROLIDINO-2-BUTYNYL]-ACETAMIDE TO PUTATIVE M2 MUSCARINIC RECEPTORS IDENTIFIED BY [3H]AF-DX 116 IN THE RAT CEREBRAL CORTEX. X. Ming* and M. Watson (SPON: F. Ehlert). Dept. of Pharmacology, University of Medicine and Dentistry of New Jersey- N. J. Medical School, Newark, N.J. 07103-2757.

BM-5, an oxotremorine analog, has been demonstrated to produce muscarinic acetylcholine receptor (mAChR) agonist like effects. However, in vivo studies reveal that it is tremorolytic. We characterized the binding of this unique partial agonist in membrane preparations of the rat cerebral cortex and heart. Binding assays for [^{3}H](-)quinuclidinylbenzilate ([^{3}H](-)QNB), a highly specific but non-subtype selective antagonist of the mAChR, the M1 selective antagonist [^{3}H]pirenzepine ([^{3}H]PZ), and the M2 selective antagonist [^{3}H]AF-DX 116 were conducted as previously described. Apparent affinity (Ki) values determined by inhibition studies ranged from 2-270nM. BM-5 produced shallow Hill values in [^{3}H](-)QNB-labeled cortical membranes, reflecting its interaction with high and low affinity sites. It had low affinity (270nM) at the [^{3}H]PZ site. Similar Ki values (20-40nM) were obtained in heart and cortex vs [^{3}H]AF-DX 116. Yet, the GTP analog guanyl-5'-yl imidodiphosphate (GppNHp) caused a greater shift to lower affinity in [^{3}H]AF-DX 116-labeled cardiac (5x) vs cortical (1.5x) M2 sites. Thus BM-5 appears to have greater affinity for M2 mAChR's and shows greater GTP coupling to cardiac M2 than cortical M2 mAChRs. Supported in part by FUMDNJ, a BRSG from NJMS, and MH-43024.

435.11

HEXAHYDRODIFENIDOL DOES NOT INDICATE THAT MUSCARINIC M1-RECEPTORS ARE HETEROGENEOUS

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Hexahydrodifenidol (HHD) has been suggested to distinguish between muscarinic M-1 receptors in the hippocampus and ganglion with pA_2 values of 5.7 and 7.9, respectively (Lambrecht et al. TIPS suppl. 22 p82, 1988). We have studied the muscarinic antagonist properties of HHD on a variety of in vitro preparations from the rat. The pA_2 against muscarine-induced depolarisations of hippocampal CA1 cells (7.4) and superior cervical ganglia (7.6) were similar. Furthermore HHD equally displaced [^{3}H]-pirenzepine binding from hippocampal and cortical membranes (pKapp = 7.85 in each tissue). Finally, it antagonised carbachol-stimulated phosphatidyl inositol turnover in hippocampus and cortex with similar pA_2 values (7.51 and 7.52). Our results with HHD do not support the proposal of multiple M-1 receptors.

435.12

AGONISTS FOR HIPPOCAMPAL M1 MUSCARINE RECEPTORS. L. T. Potter, C. A. Ferrendelli* and H. E. Hanchett*. Dept. of Pharmacology, U. of Miami School of Medicine, Miami, FL 33101.

Most of the cholinergic receptors in the cerebral cortex and hippocampus are M1 muscarine receptors. They remain after experimental cholinergic denervation and in Alzheimer's disease; and their activation clearly promotes cerebral excitation. Direct information is needed concerning which acetylcholine analogs have the highest affinity and efficacy at these receptors, in order to design optimal M1 agonists, and to test the idea that increased cerebral excitation improves memory. The binding of agonists to M1 receptors was examined by measuring competition between each agonist and 1 nM ^{3}H-pirenzepine, using rabbit hippocampal membranes from 5 mg of tissue suspended in 1 ml of 20 mM Tris buffer - 1 mM $MnCl_2$ at 25°C (Cell. Molec. Neurobiol. 8, 1-11, 1988). Computerized analyses of binding curves showed that agonists bound to a high-affinity (K_H) and low-affinity (K_L) state of M1 receptors, and that the affinity in 0.2 mM GppNHp was similar to, although usually lower than, K_L. K_L/K_H values varied from 50 or more for cis-dioxolane, oxotremorine-M and acetylcholine, to near 1 for oxotremorine and McN-A 343. There was an excellent correlation between the K_L/K_H values for different agonists at M1 receptors, and prior data for the ability of these agonists to promote cerebral excitation and phosphoinositide turnover. Thus K_L/K_H determinations appear useful for screening agonists for high efficacy. High K_H or K_L values alone did not correlate with high efficacy. The available data show that the five cholinergic agonists which have been tested for improving memory in humans (without much success) are all weak M1 agonists.

435.13

STUDIES OF GUANINE NUCLEOTIDE-SENSITIVE AGONIST-BINDING TO M1 MUSCARINE RECEPTORS IN MEMBRANES TREATED WITH N-ETHYL MALEIMIDE. D. D. Flynn and L. T. Potter. Department of Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

Studies of the agonist-binding properties of M1 muscarine receptors have been more difficult than similar studies of M2 receptors, because of the generally higher affinity of M2 receptors for agonists, and the lack of a tissue source having pure M1 receptors. Low concentrations of ^{3}H-oxotremorine-M label predominantly M2 receptors even in M1-rich tissues like the hippocampus and cortex. In order to improve assays of agonist-binding to M1 receptors, we have used rabbit hippocampal membranes treated with 0.1 mM N-ethyl maleimide (NEM), to prevent the binding of agonists to the high affinity state of M2 but not the high affinity state of M1 receptors (Mol. Pharmacol. 30, 193, 1986). Measurements of competition between oxotremorine-M and 1 nM ^{3}H-pirenzepine demonstrated that the agonist bound to high and low affinity states of M1 receptors, and that the high affinity (K_H) state was lost in 0.2 mM GppNHp. Under the conditions used (20 mM Tris-1 mM $MnCl_2$, 25°C), K_H was 20-25 nM. Under the same conditions, direct measurements of the association, dissociation and equilibrium binding of ^{3}H-oxotremorine-M revealed the same K_H for M1 receptors, and that the dissociation rate was increased 6-fold by 0.2 mM GppNHp. Thus the use of NEM-treated hippocampal membranes demonstrates an interaction between M1 receptors and an NEM-resistant G protein, and that direct measurements of agonist-binding to M1 receptors are practicable.

435.14

POSSIBLE ALLOSTERIC INTERACTION OF NICARDIPINE WITH RAT BRAIN MUSCARINIC ACETYLCHOLINE RECEPTOR SUBTYPES S.Katayama*, S.Kito and R.Miyoshi (SPON: Y.Yamamura) 3rd Dept. Int. Medicine, Hiroshima Univ. Sch. Med., 1-2-3 Kasumi, Minami-ku, Hiroshima, 734 Japan

The dihydropyridine (DHP) derivatives is a member of a group of compounds that have been termed 'calcium antagonists'. In this paper, effects of nicardipine, one of DHPs on muscarinic receptor (m-AChR) antagonist binding in the brain have been investigated. Experiments were done using ^{3}H-QNB, ^{3}H-PZ and ^{3}H-AF-DX116 to differentiate the effect on each subtype. Tissue homogenates were obtained from male Wistar-strain rats weighing 180-220g. We used P_2 fruction for ^{3}H-QNB and ^{3}H-PZ binding assay. For ^{3}H-AF-DX116 binding, tissue preparation and the binding assay were performed according to J.X.Wang (1987).

Nicardipine inhibited ^{3}H-QNB (0.2nM), ^{3}H-PZ (18nM) and ^{3}H-AF-DX 116 (30nM) binding comletely with Ki values and Hill coefficients $4.28X10^{-8}$ and 1.03, $7.36X10^{-8}$ and 0.80, and $7.87X10^{-7}$ and 0.75, respectively. Displacement curves of ^{3}H-QNB binding by nicardipine shifted to right as the concentration of ^{3}H-QNB increased. The inhibition curve became shallower at higher concentrations of the ligand. Schild plots yielded curvilinear functions. This deviation from linearity of Schild plots indicated possible allosteric interactions between calcium channels and muscarinic receptor. It was concluded that nicardipine had inhibitory effects on both M1 and M2 receptor binding.

435.15

POTENT ALLOSTERIC EFFECTS OF TETRAHYDROAMINO-ACRIDINE (THA) ON MUSCARINE RECEPTORS. B.D. Pearce,* C.A. Ferrendelli*, H.E. Hanchett* and L.T. Potter. (SPON: W.R. Loewenstein). Dept. of Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

Clinically effective concentrations of THA (0.03-0.3 μM, Summers et al. NEJM 315, 1241, 1986) readily inhibited acetylcholinesterase from rabbit hippocampal tissue in artificial CSF at 37°C, with physiological levels of substrate. Above 1 μM, THA acted on allosteric sites on M1 and M2 receptors as an antagonist, and at 10-1000 μM THA is known to block K^+ channels. Thus THA probably acts clinically as an esterase inhibitor (Neurosci. Lett., in press). Nonetheless the allosteric effects of THA are striking. Competition curves between THA and various ^{3}H-antagonists for hippocampal M1 and brainstem M2 receptors were steeper than mass action curves, and THA fully prevented radioligand binding. Blockade was unaffected when receptors were dissociated from other proteins with GppNHp or EDTA, or with EDTA and digitonin; hence THA appears to act directly on receptors. THA markedly slowed the dissociation of ^{3}H-antagonists, and was much more potent and effective than gallamine. For example, in 20 mM Tris-1 mM $MnCl_2$, 10 μM THA made the binding of ^{3}H-pirenzepine effectively irreversible, whereas 1 mM gallamine only partially slowed its dissociation. The concentrations of THA required to reduce the binding and dissociation of antagonists were equal, suggesting that all of the effects of THA are allosteric. Curiously, THA blocked the binding of the agonist, ^{3}H-oxotremorine-M, but not its dissociation. Thus THA may recognize only low affinity conformations of muscarine receptors.

435.16

Muscarinic receptor binding properties of 1,2,3,4-tetrahydro-9-acridinamine (tacrine) and other acetylcholinesterase inhibitors. S.L. Myers*, L.L. Coughenour*, D.T. Dudley, J.H. Fergus*, C.J. Spencer*, R.D. Schwarz, and B. Berghoff*. (Spon: D.K. Boyd) Pharmacology Department, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

The affinity and intrinsic activity of several acetylcholinesterase inhibitors (ACHEIs) including 1,2,3,4-tetrahydro-9-acridinamine (tacrine) were determined for muscarinic receptors in rat brain cortical membranes labeled with the muscarinic agonist ^{3}H-cismethyldioxolane (CMD) and the antagonist ^{3}H-quinuclidinyl benzilate (QNB). Ligand efficacy was indexed by the resulting antagonist/agonist (QNB/CMD) affinity ratio. In SK-N-SH cultured cells, partial to full agonists exhibited QNB/CMD values > 80. QNB/CMD ratio values for ACHEIs examined were predictive of either agonist or antagonist activity (QNB/CMD physostigmine = 1807, QNB/CMD tacrine = 8). However, all ACHEIs examined were either inactive or antagonists at stimulating PI turnover in SK-N-SH cells. The affinity of ACHEIs against ^{3}H-CMD binding correlated with their potency to inhibit acetylcholinesterase (ACHE). To determine if displacement of muscarinic binding was mediated by inhibition of ACHE, the affinities of representative ACHEIs against ^{3}H-QNB and ^{3}H-CMD binding were determined using membranes pretreated with the irreversible ACHEI, paraoxon (PAR). PAR pretreatment shifted QNB/CMD values for these ACHEIs toward 1 due to a decrease in potency to inhibit ^{3}H-CMD binding; whereas, the QNB/CMD ratio for the competitive agonist arecoline was not significantly affected. These results suggested that for those ACHEIs examined displacement of ^{3}H-CMD binding was mediated by inhibition of ACHE while displacement of ^{3}H-QNB binding was due to a direct interaction at the receptor. In addition, using this two assay binding method, PAR pretreatment distinguished ACHEI from competitive agonists.

435.17

GALLAMINE EXERTS BIPHASIC ALLOSTERIC EFFECTS AT MUSCARINIC RECEPTORS John Ellis. Neuroscience Research Unit, Department of Psychiatry, University of Vermont College of Medicine, Burlington, VT 05405.

Gallamine and a number of other compounds (verapamil, quinidine, and others) have been found to slow the rate of dissociation of labeled ligands, especially [^{3}H]N-methylscopolamine (NMS), from muscarinic receptors of heart and brain. There has been some dispute as to whether the dissociation of [^{3}H]quinuclidinyl benzilate (QNB) is subject to such allosteric regulation. We have recently found that gallamine modulates the dissociation of [^{3}H]QNB from muscarinic receptors of the heart in a biphasic manner. Low concentrations (micromolar) accelerate the rate of dissociation, while higher concentrations (millimolar) slow it; at about 0.1mM, the two effects cancel each other. Similar results are obtained with muscarinic receptors from the brainstem, but gallamine has only marginal effects on the dissociation of [^{3}H]QNB in the forebrain. On the other hand, gallamine slows the dissociation of [^{3}H]NMS to a similar extent in all three tissues (brainstem, forebrain, and heart). Furthermore, verapamil exerts only monophasic effects (slowing) on the dissociation of both [^{3}H]NMS and [^{3}H]QNB from heart receptors. The data suggest that there are multiple allosteric regulatory sites associated with muscarinic receptors. Supported by the Vermont Heart Association, the Department of Psychiatry, and the University of Vermont (BSRG).

435.18

GALLAMINE ALLOSTERICALLY MODULATES MUSCARINIC RECEPTOR BINDING AND ANTAGONIZES PHOSPHOINOSITIDE AND CYCLIC-AMP RESPONSES IN N1E-115 NEUROBLASTOMA CELLS. N.H. Lee* and E.E. El-Fakahany. Dept. of Pharmacology and Toxicology, Univ. of Maryland Sch. of Pharmacy, Baltimore, MD 21201.

Gallamine (Gall)/ 0.2 nM (^{3}H)N-methylscopolamine ((^{3}H)NMS) competition curves were shallow and best fit to a two-site model (K_H=7.6 ± 1.6 μM, R_H=40 ± 3 %; K_L=1.6 ± 0.7 mM, P<0.05). Likewise, gall/ 0.2 nM (^{3}H)N-methyl-quinuclidinyl benzilate ((^{3}H)NMeQNB) curves could be best fit to a two-site model (K_H=49 ± 4 μM, R_H=91 ± 1 %; K_L=13 ± 11 mM, P<0.05). Gall allosterically slowed the rate of dissociation of bound (^{3}H)NMS > (^{3}H)NMeQNB from muscarinic receptors (MR) in a dose dependent manner. Subtracting the allosteric component from the gall/(^{3}H)NMS and gall/(^{3}H)NMeQNB competition curves yielded a single high affinity site for gall (K_H=90 ± 8 and 62 ± 1 μM, respectively). M_1 receptor-mediated phosphoinositide hydrolysis stimulated by carbamylcholine (CBC) was antagonized by gall with a K_i value of 44 μM and a Schild slope of 1.08 ± 0.11. However, gall was less potent in antagonizing glandular M_2-mediated inhibition of cyclic-AMP formation by CBC with a K_i value of 182 μM and a Schild slope of 1.10 ± 0.08. In conclusion, gall allosterically modulates MR antagonist binding and reveals a 4-fold selectivity profile in antagonizing M_1 versus M_2-mediated responses. (Supported in part by NIH grants NS-24158, AG-07118, AG-00344.)

436.1

THE EFFECT OF LEAD ON THE VOLTAGE ACTIVATED CALCIUM CHANNEL OF APLYSIA NEURONS. D. Buesselberg*, M.L. Evans and D.O. Carpenter. (SPON: B. Jahan-Parwar). Wads. Ctr. for Labs. & Res., NYS DOH & Sch. of Pub. Hlth., Albany NY 12201.

Despite the known neurotoxic effects of lead its locus of action is unclear. We have investigated the effects of lead on calcium currents of Aplysia neurons recorded using the two electrode voltage clamp technique. The threshold concentration for effect was 1 μM lead. At 5 μM, lead reduced the peak amplitude of the calcium current by 14.7 ± 5%, at 10 μM by 26.3 ± 2.6% and at 50 μM by 57.7 ± 4%. With washing the calcium current amplitude rapidly (within 2 to 3 min) returned to about 95% of control after lead was applied for 2 min. If lead was applied for 20 min, the calcium current amplitude did not recover. When applied for 2 min, zinc did not block the calcium current at concentrations up to 100 μM. This effect of lead appears specific for the calcium channel as lead had little effect on the delayed rectifier potassium channel at 50 μM. (Supported by NIH and DAAD).

436.2

ZINC AND LEAD INTERACTIONS IN IMMATURE GUINEA PIGS. T. Rowles, C. Womac, G. Miller, A.J. Castiglioni, Jr.,* G.R. Bratton, and E. Tiffany-Castiglioni. Dept. of Veterinary Anatomy, TAMU, College Station, TX 77843.

Studies have shown an effect of lead (Pb) on neurological function and an interaction of zinc (Zn) with Pb. We have evaluated the effects of low level Pb and its interactions with dietary Zn in 3 groups of immature guinea pigs. Group 1 received standard guinea pig chow (27 ppm Zn); group 2 received egg white based diet (5 ppm Zn); and group 3 received egg white based diet (50 ppm Zn). One third of group 1 received 10 mg Pb acetate/kg body weight and one third received 20 mg Pb acetate/kg body weight. Half of groups 2 and 3 received 20 mg Pb acetate/kg body weight. All treatments were oral and lasted 18 days beginning at 14 days of age. All groups showed increased blood and tissue Pb levels in Pb-treated animals compared to controls, but the cerebral levels of Pb were less than blood levels. Even without concentration of Pb in the cerebrum, the mean maximum diameters of the astroglia were increased in the Pb-treated animals. Neither Pb nor Zn had an effect on absolute brain weight or brain weight to body weight ratios. Dietary Zn levels did not affect levels of Cu, Zn, or Fe in the brain. Further evaluation of the significance of an increase in astroglial diameter without concentration of Pb in the cerebrum is underway. Funded by Center for Energy & Mineral Resources, TAMU and EPA R811500.

436.3

DIFFERENTIAL TOXIC EFFECTS OF CIS-PLATIN AND TRANS-PLATIN ON CHICK DORSAL ROOT GANGLION CELLS IN VITRO. K.S.Blisard and S.L. Rogers. Research Service (151), Veterans' Administration Medical Center, and Departments of Pathology and Anatomy, University of New Mexico Medical School, Albuquerque, NM 87112

Cis-diamminedichloroplatinum (II) (cis-platin) is a useful cancer chemotherapeutic agent. An important side effect of this drug in humans is the development of a peripheral neuropathy, the mechanism of which is not known. As a model system, we have administered cis-platin to cultured chick embryonic dorsal root ganglion cells. At doses of 50-100 ug/ml, the drug was toxic to cultures in 6 hours, and changess were seen in both neurons (detachment) and non-neuronal cells (vacuolation). After 24 hours, there was extensive cell death. The trans isomer of the drug, trans-platin, was much less toxic to cultures. At concentrations of 75 or 150 ug/ml, very minimal changes were seen in cultures which had been exposed to trans-platin for 6 or even 24 hours. This was significantly different from the observations with cis-platin, and suggests that a steric interaction, which is different for the two isomers of the drug, is important in the toxicity of cis-platin.

436.4

FREE AMINO ACIDS IN PLASMA AND BRAIN AFTER CHRONIC MANGANESE INTAKE. E. Bonilla, A.L.N. Prasad*, J.O. Dávila*, A. Arrieta* and R. Villalobos*. Instituto de Investigaciones Clínicas, Universidad del Zulia and INBIO-MED-FUNDACITE, Aptdo 376. Maracaibo-Venezuela.

The present study was conducted to determine the changes in the concentrations of free amino acids in plasma and brain after chronic manganese intake.

Male Sprague-Dawley rats weighing 200-250 g were treated with 1 mg of Mn per ml of drinking water. Both the manganese-treated and the control group had unrestrained water access. At eighth month, 11 animals of each group were sacrificed by decapitation. The brains were extracted immediately and placed at 4°C to dissect the striatum and frontal cortex. Prior to the sacrifice a blood sample was obtained from each rat by cardiac puncture. The analysis of the amino acids was performed by HPLC.

The growth rate of manganese intoxicated rats was normal. Brain manganese concentrations increased significantly. The means ± S.E. of the manganese content (expressed in ug/g dry weight) at the eighth month were: a) frontal cortex: 1.7 ± 0.3 in controls and 5.1 ± 0.4 in Mn-loaded rats; b) striatum: 1.8 ± 0.1 and 3.2 ± 0.2. The mean ± S.E. daily water intake during the eighth month for each rat was: 60.1 ± 0.7 ml for controls and 54.0 ± 0.5 for the Mn-treated rats. The difference was significant ($p < 0.001$).

No changes were observed in the levels of plasma amino acids in manganese-treated rats. Similar results were observed in the striatum and frontal cortex. In the light of these results it is safe to assume that the chronic manganese intake of 1 mg Mn per ml of drinking water did not affect either the gastrointestinal absorption of the amino acids nor their passage through the blood-brain barrier.

436.5

REVERSAL OF ALUMINUM-INDUCED BEHAVIORAL DEFICITS IN THE RAT BY DFO. D.J. Connor, L.E. Harrell and R.S. Jope. Dept. Psychology, Neurology and Pharmacology, Univ. of Ala., Birmingham, AL. 35294.

Administration of aluminum sulfate in the drinking water of male Sprague-Dawley rats for thirty days resulted in a reduction in the number of days to reach extinction criterion on a passive avoidance task (38% of control level). Controls yoked by fluid consumption to the treated animals demonstrated that the behavioral deficit was not due to nonspecific effects caused by lower fluid consumption. No change in vertical or horizontal open field activity was evident after the aluminum treatment. Partial improvement of the deficit was produced by replacement of the aluminum sulfate solution with tap water two weeks prior to testing ($p<0.05$). Replacement with tap water in combination with injection (i.p.) of the aluminum chelator desferoxamine (DFO: 75 mg/kg or 30 mg/kg) returned the performance of the aluminum-treated animals to control levels in a dose-dependent manner. DFO injection of control animals did not affect their activity levels or extinction on the passive avoidance task. These results indicate that the behavioral impairment is a specific, toxic effect of aluminum administration and that it can be reversed by administration of an aluminum chelator.

436.6

TRIETHYL TIN: EFFECTS ON CHOLINERGIC SYNAPTIC TRANSMISSION. R.D. Laurie*, G.P. Cooper, D.J. Minnema* and R. Greenland*. Dept. of Environ. Health, Univ. of Cincinnati Sch. Med., Cincinnati, OH 45267.

The in vitro sciatic nerve-sartorius muscle preparation of the frog (Rana pipiens) and isolated cholinergic synaptosomes obtained from rat brain were used to assess the effects of triethyltin chloride (TET) on "spontaneous" acetylcholine (ACh) release, depolarization-evoked ACh release, nerve terminal action potential (AP) latency and amplitude, and muscle resting membrane potential (Em). TET (1-100 μM) was incorporated into appropriate salt solutions and the preparations continuously superfused. In the frog TET produced a steady decline in Em, the rate and extent of which was dose-related. When tetraethylammonium chloride (TEA) was used to replace NaCl both the rate and degree of depolarization was reduced, while choline chloride replacement had little effect. TET increased the AP latency and decreased its amplitude. In both the frog and rat preparations spontaneous ACh release was initially increased but rapidly declined during TET exposure. In synaptosomes depolarization-evoked release was unaffected by TET; in the frog both the endplate potential and miniature endplate potential amplitudes were decreased more than could be accounted for by the fall in muscle Em. These studies indicate that TET has no direct effect on membrane ion channels but probably causes an increase in the muscle and nerve membrane leakiness to K^+ and possibly other monovalent ions. Support: NIEHS 03992.

436.7

MECHANISM OF TRIETHYLTIN BROMIDE-INDUCED NEUROTOXICITY. B.E. Morton, E.F. Block* and F. Taketa*, Univ. of Hawaii, Honolulu, HI 96822 & Med. Coll. of Wis. Milwaukee, WI 53226

Respiration is diminished in brain slices from rats injected with triethyltin (TET) derivatives (Biochem J. 119 95-102, 1970). Pyruvate dehydrogenase activity is also reduced in brain homogenates or mitochondria prepared from TET-intoxicated rats (FASEB J. 2 A 1373, 1988). We have now inquired whether TET affects glucose metabolism in live, conscious rats by analyzing its influence on the uptake of 2-deoxyglucose (2-DG) in brain. [14C]-2-DG (125uCi/Kg) was injected i.p. at timed intervals after i.p. injection of TET into groups of rats. Animals were sacrificed 45 min later and their brains removed for sectioning and autoradiographic analysis (J. Neurochem. 78 897-916, 1977). In addition, we investigated possible mechanisms by which TET acts by evaluating neurotransmitter receptor agonists and antagonists as potential antidotes for TET-neurotoxicity. Ligands, acting on ionophore-regulating inhibitory and excitatory receptors were administered by i.p. injection prior to TET administration and their influence on the course of development of neurotoxicity was monitored. Statistically significant reductions in global and regional uptake of 2-DG were found in brains of TET-treated rats compared with control animals. Preadministration of the putative antidotes resulted in complex changes suggesting that some of the effects of TET can be modified by ionophore regulation. [Supported by grants from the NIH (ES04005) and The Univ. of Hawaii Foundation.]

436.8

TRIETHYLTIN NEUROTOXICITY IS NOT MEDIATED BY EXTRACELLULAR CA^{2+}. Y.L.T. Ting, S.B. Fountain, T.J. Teyler. N.E. Ohio Univs. Coll of Med. Rootstown, OH 44272.

To assess the role of extracelluar Ca^{2+} in synaptic suppression by triethyltin (TET) (Fountain et al., Neurotoxicol. Teratol. in press), we exposed in vitro hippocampal slices to TET in a Ca^{2+}-free environment. Slices were prepared using standard techniques and maintained at the interface of a pool of artificial CSF and an O_2/CO_2 atmosphere. Recording and stimulating electrodes were positioned in the Schaffer collaterals. When a stable waveform was recorded, an input-output (I/O) profile was obtained by administering a series of increasing stimulus intensities. Following the baseline I/O, Ca^{2+} was removed from the pool by washout with Ca^{2+}-free medium. After 1 hr, the EPSP could no longer be evoked, and at this time any tissue-bound CA^{2+} released after the first exchange was removed with a second CA^{2+}-free washout. Following the second Ca^{2+}-free exchange, slices were exposed to 10 uM TET. Evoked responses were recorded for 2 hrs at 5 min intervals, only to be interrupted at 1 and 2 hr post-TET exchange for I/O assessments. After the I/O assessment at 2 hr postexposure, TET was washed out of the pool using Ca^{2+}-free medium, followed by an exchange of Ca^{2+}-bearing medium. Ca^{2+} replacement did not produce recovery of the EPSP. Thus, TET-induced synaptic suppression is probably not mediated through extracellular Ca^{2+}. It is possible that attenuation of evoked synaptic responses reflects TET-induced gradual depolarization of hippocampal neurons by uncoupling oxidative phosphorylation. (Supported by EPA, ONR and NIH)

436.9

TRIMETHYLTIN-INDUCED NEUROTOXICITY MAY NOT BE MEDIATED THROUGH AN EXCITOTOXIC MECHANISM. J.P. O'Callaghan, D.M. Niedzweicki, M.E. Gilbert, L.P. Miller and P. Ornstein.* U.S. EPA and Northrop Services, Res. Tri. Pk., NC 27711, VA Medical Ctr. and Georgetown University, Washington, D.C. 20422 and Eli Lilly and Co., Indianapolis, IN 46206.

Systemic administration of trimethyltin (TMT) to the rat results in a pattern of hippocampal damage similar to that seen after local or systemic administration of excitotoxic analogues of glutamate. We used agents known to inhibit glutamate receptor-mediated effects to determine the role of glutaminergic pathways in TMT neurotoxicity. Acute administration of TMT resulted in a loss of hippocampal pyramidal cells (CA1 & CA3-4). Autoradiography revealed decreased NMDA, quisqualate (QA) and kainate (KA) receptors; QA and KA receptors were most affected. Systemic or intrahippocampal (IH) injections of TMT in combination with six daily injections (s.c. or IH) of the NMDA receptor antagonists, ketamine, AP7 and MK-801, did not alter the neurotoxic effects seen after TMT alone, as evidenced by histology and assays of the neuron-specific and astrocyte specific proteins, synapsin I and GFAP. Activation of KA receptors produce seizure activity but daily IH EEG recordings failed to reveal seizure activity in TMT-treated rats. Moreover, daily administration of phenobarbital did not affect TMT-induced neurotoxicity. The data argue against a role for NMDA and KA receptors as mediators of the neurotoxic effects of TMT but do not rule out involvement of QA receptors.

436.10

AUTOMAINTAINED REVERSAL LEARNING IN RATS: PARAMETRIC ANALYSIS OF THE EFFECTS OF TRIMETHYLTIN (TMT). P.J. Bushnell. Neurotox. Div., US EPA, RTP, NC 27711.

Automaintenance involves Pavlovian conditioning in that pairing salient stimuli elicits specifiable behavior from animals. Rats received food (UCS) after a 15-sec extension of one of two levers (CS^+) into an operant chamber; extension of a second lever (CS^0) was uncorrelated with food. Responding to each lever was recorded across ten 10-trial blocks of each daily test, but did not affect food delivery. Rats responded preferentially to the CS^+, with discrimination ratios (DRs) approaching 1.0. Reversal (R) of the locations of CS^+ and CS^0 caused redirection of responding to the new CS^+. By R10, all rats reversed reliably within 100 trials. Asymptotic R performance was disrupted by TMT (7 mg/kg, iv) when a 2- or 4-sec delay separated the CS^+ from the UCS. The disruption included decreased responding to the CS^+ and increased responding to the CS^0, without change in total responding. The reduction in CS^0 responding across trials was significantly slower for TMT rats than for controls, and was highly correlated with degree of damage to the hippocampus. Hyperbolic functions fitted to R curves (ΔDR over trials) showed that hippocampal damage correlated significantly with DR asymptote but not with the trial number at which the DR was half-maximal. This procedure permits quantification and characterization of learning deficits induced by neurotoxic chemicals, and may help identify CNS substrates involved in learning.

436.11

EFFECT OF AMMONIA ON THE BENZODIAZEPINE (BZD) RECEPTOR IN CULTURED ASTROCYTES. I. Ducis*, L.O.B. Norenberg*, and M.D. Norenberg. Lab. Neuropath., Vet. Adm. Med. Ctr. & Univ. of Miami Sch. Med., Miami, FL 33101.

There is evidence that the BZD receptor may be involved in ammonia/hepatic encephalopathies. Since astrocytes appear to be critically involved in these disorders, we studied the effect of ammonia on the BZD receptor in primary astrocyte cultures. Astrocytes were obtained from neonatal rat cortices, and after 2 weeks, half of the cultures were treated with 0.5 mM dibutyryl cyclic AMP (dBcAMP). Scatchard analysis of the binding of ^{3}H-Ro5-4864 to astrocyte homogenates in the presence of 2 and 5 mM NH_4Cl showed a significant decrease in Kd: 27% and 32%, respectively ($p<.05$) in cells that had not been maintained with dBcAMP. However, no significant increase in binding affinity was observed in the presence of 10 mM NH_4Cl. No significant change in Kd nor Bmax was found at any NH_4Cl concentration when cells were maintained with dBcAMP. Homogenates from non dBcAMP-treated cells, however, showed a 14% decrease ($p<.05$) in receptor number after treatment with 10 mM NH_4Cl although no decrease was observed with 2 and 5 mM NH_4Cl. The latter results are consistent with the observation that dBcAMP has a protective effect on the ammonia-induced morphological changes observed in cultured astrocytes. Our findings furthermore suggest that some of the BZD receptor changes found in models of hepatic encephalopathy may occur on astrocytes and thus implicate the astrocyte BZD receptor in the pathogenesis of hepatic encephalopathy.

436.12

AMMONIA INDUCED DECREASE IN GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IN CULTURED ASTROCYTES. M.D. Norenberg, L.O.B. Norenberg*, and J.T. Neary. Vet. Adm. Med. Ctr. & Univ. Miami Sch. Med., Miami, FL 33101.

Previous immunocytochemical and quantitative studies have indicated a specific reduction in GFAP in human hepatic encephalopathy (HE). Since ammonia is a prime candidate as the etiologic factor in HE and since astrocytes appear to be the principal target of ammonia toxicity, we investigated the direct effect of ammonia on GFAP in astrocytes in primary culture. Astrocyte cultures were prepared from newborn rat cortices. Some cultures were treated (day 14) and maintained in 0.5 mM dibutyryl cyclic AMP (dBcAMP). Cultures (5 to 7 weeks old) were treated with 2 and 10 mM NH_4Cl for 1 or 4 days. Cells were homogenized in SDS/EDTA/Tris buffer and supernatants were prepared. GFAP was measured by enzyme linked immunosorbent assay with porcine anti-GFAP and rabbit anti-GFAP antisera kindly supplied by Dr. L. Eng, Palo Alto, CA. No significant differences in GFAP content were observed in cultures exposed to 2 or 10 mM NH_4Cl for 1 day. However, treatment of cultures for 4 days with 10 mM NH_4Cl showed a $39\pm7\%$ decrease in GFAP (mean$\pm$SEM, n=6; $p<0.05$). The effect was dose-dependent as a smaller decrease in GFAP ($23\pm3\%$) occurred with 2 mM NH_4Cl. No significant fall in protein was noted. Astrocytes treated with dBcAMP were more resistant to ammonia; a $24\pm3\%$ decrease was observed upon treatment of these cultures with 10 mM NH_4Cl. The latter observation is consistent with the protective effect of cyclic AMP on ammonia-induced gliotoxicity. These results indicate that ammonia causes a reduction in GFAP and may be the principal factor responsible for the loss of GFAP in human HE.

436.13

AN ELECTROPHYSIOLOGICAL STUDY OF HYDROGEN SULFIDE TOXICITY IN FROG SYMPATHETIC GANGLIA. S.B. Kombian*, J. Zidichouski*, M. Kehoe* and R.J. Reiffenstein, Pharmacology Dept., Univ. of Alberta, Edmonton, AB. Canada T6G 2H7

Hydrogen sulfide (H_2S) and its alkali salts are very toxic. H_2S has caused many industrial deaths, apparently by paralysis of medullary respiratory drive. Few electrophysiological studies of its toxicity have been done (Warenycia et al., Soc. Neurosci. Abstr. 13, 94, 1987). Using sucrose gap recording, we studied the effect of NaHS (1.8 mM) on the Na^+/K^+ pump (K^+ - activated hyperpolarization), and on agonist-induced conductance changes in sympathetic ganglia of Rana pipiens. Muscarine-induced (10 μM) hyperpolarization was potentiated to 108.8 ± 5.2% of control ($p<0.05$, n=14), and the hyperpolarizing effect of 1 μM epinephrine to 117.1 ± 6.4% of control, ($p<0.05$,n=7). NaHS did not affect depolarization due to 10μM nicotine (97.2 ± 6.6% of control, $p>0.6$, n=7). The Na^+/K^+ pump activity was not initially affected by NaHS (117 ± 12% of control, $p>0.4$, n=7), but removal of NaHS potentiated the response. A consistent NaHS-induced depolarization was observed (2.75 ± 0.5 mV, $p<0.001$, n=23). The lack of significant attenuation of these diverse responses does not support a rapid anti-metabolic action of NaHS. Supported by Alberta Community and Occupational Health.

436.14

THE EFFECTS OF HYDROGEN SULFIDE EXPOSURE ON AMINO ACID LEVELS IN THE DEVELOPING RAT CEREBRUM AND CEREBELLUM. R.S. Hannah*, S.H. Roth and J. Hayden*. Depts. of Anatomy and Pharmacology and Therapeutics, University of Calgary, Calgary, Alta, Canada.

Humans can be exposed to various chronic levels of the environmental pollutant, hydrogen sulfide (H_2S). The exposure occurs both in the work place and by living in proximity to industries which produce H_2S. Since H_2S intoxication produces a variety of biochemical changes in the mature CNS of laboratory animals, it is essential to examine the effects on the developing CNS which is extremely sensitive to environmental disturbances. Timed-pregnant Sprague-Dawley rats were exposed to H_2S (75 ppm) for seven hours per day from Day 6 postcoitus until Day 21 postnatal (pn). Entire litters were euthanized on Days 7, 14 and 21 pn. The cerebrum (anterior to the optic chiasm) and cerebellum were removed and stored at -70°C. The putative amino acid neurotransmitters, aspartate, glutamate, glycine, taurine and GABA were quantitated using precolumn fluorescence derivatization with OPT and reversed-phase high performance liquid chromatography. In both regions, aspartate, glutamate and GABA were significantly reduced below control levels by Day 21 pn. Taurine which was initially elevated returned to control levels by Day 21 pn. Glycine levels were unaltered following exposure to H_2S. (Supported by Alberta Occupational Health and Safety).

436.15

BRAIN CATECHOLAMINE LEVEL ELEVATIONS AFTER H_2S. R.J. Reiffenstein, M.W. Warenycia & S.B. Kombian*. Dept., of Pharmacol., Univ. Alberta, Edmonton, Canada T6G 2H7.

H_2S intoxication leads to loss of central respiratory drive. Since catecholamines (CAs) are important for neural control of respiration, regional CA levels were examined after H_2S. Male SD rats, (≈300g) were given saline, 10 or 30mg/kg NaHS (0.66 or $2xLD_{50}$) ip. and killed at 2 min. Brainstem, cerebellum, hippocampus, striatum and cortex were weighed, sonicated in 0.1N $HClO_4$ and supernatants analyzed for CA content by HPLC with EC detection. Results are given in μg/g as the $\bar{X}$±SEM; (N=5) for dopamine(DA), noradrenaline(NA) and adrenaline(A). None changed at 10mg/kg NaHS, nor cortex or cerebellum at 30 mg/kg NaHS. *=$p<0.05$; (Duncan's Test) C=controls; T=30mg/kg NaHS.

		DA	NA	A
BRAINSTEM(B)	(C)	0.57±0.06	0.90±0.13	0.43±0.03
	(T)	1.61±0.40*	1.82±0.12*	1.26±0.08*
HIPPOCAMPUS(H)	(C)	0.81±0.19	1.09±0.10	0.51±0.07
	(T)	0.98±0.20	2.94±0.73*	1.82±0.30*
STRIATUM(S)	(C)	6.60±0.48	1.78±0.06	1.13±0.18
	(T)	6.37±0.34	2.33±0.33*	1.89±0.52*

Both NA and A increased in B, H and S; DA only in brainstem. Changes in CAs after H_2S indicate either increased synthesis or decreased catabolism, possibly by inhibition of MAO. (Supported by Alberta Community and Occupational Health).

ALZHEIMER'S DISEASE: NEUROPATHOLOGY

437.1

PREFERENTIAL LOCALISATION OF COPPER ZINC SUPEROXIDE DISMUTASE IN THE INJURED HIPPOCAMPAL NEURONS IN ALZHEIMER'S DISEASE. I.Ceballos*1, F.Agid*2, A.Delacourte*3, E.Hirsch*2, P.M.Sinet*1, and Y.Agid*2 (SPON:B.Zalc).1:CHU Necker-EM 75015 PARIS, 2:U289 INSERM CHU Pitié-Salpétrière 75013 PARIS, 3:U16 INSERM Faculté Médecine 59045 LILLE.FRANCE

The distribution of cells containing copper zinc superoxide dismutase (CuZnSOD) protein and mRNA was determined by using immunohistochemistry and in situ hybridization in hippocampi and in various cortical regions from control human brain. Results obtained with these two methods are similar and demonstrate that pyramidal neurons and granule cells of hippocampus contain high amounts of CuZnSOD protein and mRNA.

In the hippocampus of an Alzheimer's patient, successive immunostaining by antiCuZnSOD and antipaired helical filaments (PHF) antibodies show that antiPHF label the same neurons as antiCuZnSOD antibodies. Thus, pyramidal neurons which are succeptible to degenerative processes in Alzheimer's disease (AD) contain high amount of CuZnSOD protein. This might indicate that biochemical pathways leading to $O_2^{\cdot-}$ generation are specially active in these neurons, requiring a high CuZnSOD content to eliminate these radicals.

Quantitative analysis by in situ hybridization are in progress to evaluate the transcription of CuZnSOD gene in Alzheimer's disease brain.

437.2

A REEXAMINATION OF ALUMINUM IN ALZHEIMER'S DISEASE. R. W. Jacobs, T. Duong, R. E. Jones* and A. B. Scheibel, Anatomy and Psychiatry Depts. and the Brain Research Institute, UCLA Med. Center, and Geology Dept., Los Angeles, CA 90024.

This study reassesses the presence of aluminum in Alzheimer's disease (AD) as previously reported by Perl and Brody (1984) and Candy et al. (1986) using **X-ray microprobe** analysis. Hippocampal and neocortical tissue samples were obtained from six clinically diagnosed and neuropathologically confirmed cases of AD (5 females/1 male; ages 74-102 yrs) and five controls (1 female/4 males; ages 43-74 yrs) with autolysis times between 7 and 30 hrs (mean of 13.5). To demonstrate senile plaques and neurofibrillary tangles, alternating cryostat sections (40 μm) were treated with thioflavin-S, Congo red, Bielschowsky's silver stain, and by immunohistochemistry for amyloid P-component (Scheibel et al., Soc. Neurosci. Abstr., Vol. 13, Part 2, p.1152, 1987). After identification with light microscopy, these lesions and the nuclei of tangle-bearing neurons were viewed under the scanning electron microscope and microprobed (beam strengths: 10-15 KeV, 0.5-1.5 nA; probe times: ≥150 sec.; beam diameter= 1 μm) to determine their chemical composition. Control tissue was identically treated and the cytoplasm/nuclei of the neurons were similarly microprobed. Virtually **no aluminum or aluminosilicates were found in the AD cases** or in the controls. There was also no unique distribution pattern or "signature" of chemical elements to distinguish the hallmark lesions of AD. We conclude that aluminum does not appear to play a significant role in the manifestation of AD. Further investigations should settle this important question.

437.3

IDENTIFICATION OF TYPE 3 PROTEIN KINASE C IN NT2/D1 EMBRYONAL CARCINOMA CELLS. K.L.Leach*, V.A.Ruff*, E.A.Powers*, J.K.Mayo* and I.Abraham*.(SPON: D.Hyslop). Dept. of Cell Biology, The Upjohn Company, Kalamazoo, MI 49001.

Alzheimer's disease is characterized by the occurence of neurofibrillary tangles and neuritic plaques. The exact protein composition of tangles is not known, but the presence of abnormally phosphorylated proteins has been demonstrated. This suggests that changes in either kinase or phosphatase systems may be involved in the development of these neuropathological changes. There are currently no established cellular models that mimic the Alzheimer pathology. However, the human embryonal carcinoma cell line, NT2/D1, has been suggested to be a candidate for such a model (Cole, G.M. and Timiras, P.S. in Model Systems of Development and Aging of the Nervous System, 1987). Treatment with 10^{-5} M retinoic acid induces neuronal differentiation of these cells, resulting in neurite outgrowth (Andrews, P.W. Developmental Biology 103:285, 1984). We are using these cells as a potential model system for studies on the regulation of protein kinases in cells with neuronal characteristics.

We have produced type-specific antibodies to brain PKC. These monoclonal antibodies (mAbs) are specific for Type 3 PKC in both ELISA and immunoblot assays. The mAbs can be divided into 2 classes based on their ability to recognize either the 45 kDa catalytic domain or the 35 kDa regulatory domain fragment of PKC. Each of the mAbs inhibits phosphorylation of histone or lipocortin by PKC. Only those mAbs that recognize the 35 kDa regulatory domain inhibit phorbol ester binding. This functional inhibition as well as the type specificity suggests that these mAbs can be used to study the activation and regulation of Type 3 PKC. Using these mAbs, we are investigating changes in PKC expression in NT2/D1 cells. The presence of Type 3 PKC was shown on Western blots using lysates prepared from undifferentiated NT2/D1 cells. The primary reactivity of the mAbs was with the 82 kDa PKC. We did not find any evidence of PKC fragments, since we did not observe cross-reactivity with smaller molecular weight species. Our results suggest that the NT2/D1 cell line may be a good model for studying kinase regulation in neuronal differentiation.

437.4

QUANTITATIVE ASSESSMENT OF SYNAPTIC DENSITY IN ALZHEIMER'S DISEASE. S.W. Scheff, S.T. DeKosky and D. Price*. Depts. Anatomy & Neurobiology and Neurology, Lexington VAMC and Sanders-Brown Research Center on Aging, University of Kentucky, Lexington, KY 40536.

Morphological studies of the brain's of patients with Alzheimer's Disease (AD) have reported significant changes in the neuron number and the dendritic structure of cortical neurons. These data, obtained from autopsy material, suggest a decrement in the number of synaptic contacts as a consequence of the disease. The present study examined the density of synaptic contacts in the frontal cortex (Brodmann's area 9) from autopsy-proven AD patients and age-matched, post-mortem matched controls taken within 13 hours of death. The brain tissue was prepared for ultrastructural analysis using immersion fixation and standard osmium tetroxide postfixation. Stereological methods were employed to ascertain synaptic density in cortical lamina III and V. Individuals with AD exhibited a highly significant reduction in synaptic density in both laminae as compared to controls. Mean synaptic apposition length was significantly larger in AD patients and was highly correlated with individual synaptic density in both control and AD brain. This suggests a possible compensatory mechanism. These results could not be accounted for by differences in postmortem interval or by the mean age of the groups. Coupled with the reported decline of ascending (extrinsic) cortical inputs and concomitant intrinsic neuronal loss, these results indicate that AD cortex is unable to maintain normal synaptic density and shows a diminished regenerative response. (Supported by ADRDA IIRG-87042, NIH AG05119 & NS21541 and the VA Research Service.)

437.5

FOCAL SYNAPTIC LOSSES IN THE HIPPOCAMPAL COMPLEX IN ALZHEIMER'S DISEASE. J.E. Hamos, L.J. DeGennaro and D.A. Drachman. Dept. of Neurology, Univ. of Mass. Med. Ctr., Worcester, MA 01655.

To understand the extent and location of neuronal losses necessary to produce dementia in patients with Alzheimer's Disease (AD), we first studied the immunohistochemical staining of synaptic terminals in postmortem tissue. Antibodies against two proteins found in synaptic terminals - synapsin I and synaptophysin - were used to label these structures in the hippocampal complexes of 8 patients with autopsy-proven AD and 9 non-demented control subjects. All AD patients showed a striking decrease in staining in the outer half of the molecular layer of the dentate gyrus when compared with this layer in the control brains which exhibited a uniform density of staining in both inner and outer halves. The altered density of immunoreactivity, suggesting a focal synaptic loss in this area, was independent of the presence of neuritic plaques and neurofibrillary tangles in the immediate vicinity. An additional patient with progressive degenerative dementia clinically, but without plaques or tangles, also showed depleted staining in the dentate gyrus. The pathogenetic significance of these synaptic losses in producing clinical dementia remain to be determined.

437.6

SITES OF EARLY ALZHEIMER-TYPE PATHOLOGIC CHANGES IN ENTORHINAL CORTEX AND HIPPOCAMPUS VISUALIZED BY ALZ-50 IMMUNOCYTOCHEMISTRY. B.T. Hyman and G.W. Van Hoesen. Depts. of Neurology and Anatomy, Univ. of Iowa, Iowa City, IA 52242

The presence of a few neurofibrillary tangles (NFTs) in the brain of a "normal" elderly person is not an unusual pathological finding. These lesions may represent a preclinical or early stage of Alzheimer's disease (AD), and it is possible that the sites of AD pathology in these cases are the initial lesions of the disease. To explore this, we have reviewed our brain collection for cases, regardless of clinical history, in which minimal or no NFTs were present. The topography of pathologic lesions was noted using both thioflavin S and Alz-50 immunocytochemistry, an antibody that likely recognizes neurons in a "pre-NFT" stage. In these cases, NFTs and Alz-50 positive neurons were found in layer II of entorhinal cortex, the continuation of this lamina into layer III of perirhinal cortex, and the subicular/CA1 area. In some cases these specific cells were positive for Alz-50, although no NFTs were present. These are all lamina and cytoarchitectural areas that are specifically and severely affected in all cases of proven AD that we have examined. Our results are consistent with the hypothesis that these neurons are vulnerable to Alzheimer pathologic degeneration, and may be among the first sites of pathology in the brain. (We thank P. Davies for generously providing Alz-50; supported by the Mathers Foundation, and NS 14944 and PO NS 19632).

437.7

STELLATE CELLS IN LAYER II OF THE ENTORHINAL CORTEX ARE PRONE TO NEUROFIBRILLARY TANGLE FORMATION IN AGING AND ALZHEIMER'S DISEASE. T. Duong, T. Abebe* and A.B. Scheibel. UCLA Departments of Anatomy, Psychiatry and the Brain Research Institute, Los Angeles, California 90024.

We have studied the layer II neurons in the entorhinal cortex of Alzheimer's (5 female / 5 male; age = 66 - 102 years) and normal patients (5 male; age = 61 - 75 years). All clinical diagnoses of Alzheimer's disease were neuropathologically confirmed. The postmortem time varied from 7 to 15.5 hours. Cryostat sections (40 μm) were processed by routine histological stains and peroxidase-antiperoxidase immunocytochemistry directed against human amyloid P-component (Scheibel et al., Soc. Neurosci. Abstr., Vol.13, Part 2, p.1152, 1987). In man, the entorhinal cortex (Brodmann's field 28) is located in the anterior portion of the parahippocampal gyrus. Neurons in layer II of the entorhinal cortex consist of alternating clusters of small (6-12 μm) and medium (25-30 μm) neurons. The medium neurons are stellate cells and they congregate in characteristic groupings of variable size (300-600 μm in diameter). In normal aging, occasional neurofibrillary tangles (NFTs) can be demonstrated in these islands of stellate cells. The NFT formations extend from the distinctive "flame-shaped" or "whorled" neuronal somatas into the proximal dendrites. **In patients diagnosed with Alzheimer's disease, nearly all the stellate neurons in layer II of the entorhinal cortex are converted to neurofibrillary tangles. NFTs in both Alzheimer's and normal patients label positively for the antibody to human amyloid P-component.**

437.8

New Pathological Features of Alzheimer's Disease (AD) Described with the Monoclonal Antibody Tor 23. P.D. Kushner, D.T. Stephenson*, and C. Greco* Pacific Presbyterian Medical Center, San Francisco, CA 94115.

Pathological hallmarks of AD, plaques, tangles, and specific cholinergic neuronal loss, have not indicated any unusual cell surface dysfunctions. This is curious in view of recent data that the amyloid precursor gene encodes a polypeptide with a transmembrane spanning sequence. We are studying neuronal surface molecules utilizing monoclonal antibodies made to ray cholinergic nerve terminals. The antibody Tor 23 binds the apparent limiting membrane of a discrete population of neurons of the human cortex and a subpopulation of subcortical astroctytes (Stephenson et al., these abstracts). In this study we examined cortical regions from AD cases to determine if the epitope defined by Tor 23 was present, absent, or altered.

Examination of the cortex from six AD cases by immunohistochemical analyses revealed distinct differences in the distribution of Tor 23 binding relative to that of control cortex. In AD, there were fewer Tor 23 positive neurons and the immunopositive astrocyte was absent. Because a particular epitope was localized, we cannot be certain that cells were lost: they could have lost immunoreactivity. Given its pivitol surface location and its conservation within the vertebrate species, the fallout of the epitope alone may be significant. Because reactive gliosis is present in AD cortex, our finding of the loss or transformation of a glial cell is unique and warrants further examination. Funded by the Department of Health Services, California.

437.9

Tor 23 Defines a Highly Conserved Neuronal Surface Epitope Present on Rare Neurons of the Human Cortex. D.T. Stephenson*, S. Wright*, and P.D. Kushner. (SPON: L.C. Fritz) Pacific Presbyterian Medical Center, San Francisco, CA 94115.

Tor 23, a monoclonal antibody from a library made to Torpedo synaptosomes (JNC 43 775-786, 1984) binds the external membrane of a discrete set of rat CNS neurons (J. Neurosci., 1988). The epitope defined by Tor 23 is conserved in the human nervous system (Muscle & Nerve 11 10-20, 1988) and is present on the external surface of a discrete subset of neurons (Mol. Br. Res. 2 271-275, 1987).

The present in situ findings within the iso- and allocortices support the distribution of Tor 23 to the apparent limiting membrane of rare and select neurons. In the frontal, occipital, parietal and motor cortical areas Tor 23 outlined neurons of laminae 2-3. These neurons were medium to large sized, rounded, and non-pyramidal. In the hippocampus, immunopositive neurons were large and were usually located just external to the pyramidal cell layer. In addition to the stained neurons, Tor 23 bound astrocytic cells of the subcortical white matter. Co-staining with GFAP indicated that only some subcortical astrocytes were Tor 23 positive. The antigen appears to be a polypeptide by immunoblot analysis.

The conservation of an epitope from elasmobranchs to humans is evidence in favor of an important function. Its position on the neuronal external surface suggests it may play a dynamic and vital role in developing and maintaining the complex neuronal milieu of the human cortex. Its appearance on a population of astrocytes may reflect a neuronal-glial interaction among a highly specialized neural subpopulation.

437.10

A NEW MONOCLONAL ANTIBODY WHICH RECOGNIZES EXTRACELLULAR NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE (AD). C.L. Joachim,* K.S. Kosik* and D.J. Selkoe (SPON: S. Khoshbin). Center for Neurologic Diseases, Brigham and Women's Hosp. and Harvard Med. Sch., Boston, MA 02115.

A new monoclonal antibody is described. Partially purified amyloid protein isolated from AD meninges was used as immunogen in mouse. In formalin-fixed paraffin-embedded tissue sections from cases of AD, this antibody labels extracellular ("ghost") neurofibrillary tangles (NFT) but not intracellular NFT. Some amyloid plaques are very lightly labeled, and this immunostaining is enhanced by pretreatment of tissue sections with 88% formic acid. Formic acid pretreatment does not alter labeling of extracellular NFT and does not cause intracellular NFT to become immunoreactive. On frozen sections of brain tissue, this antibody labels nuclei of all cells. This antibody does not recognize bovine tau protein or purified glial fibrillary acidic protein on Western blots, nor does it recognize the 28 residue synthetic beta amyloid peptide on dot blots. No protein bands are labeled on Western blots of Triton-soluble proteins from homogenates of AD or control cerebral cortex. Studies are under way to identify the antigen(s) recognize by this antibody. Our hypothesis is that proteins immunologically cross-reactive with the beta amyloid protein may coat the filaments of extracellular tangles, and thus be recognized by this antibody.

437.11

A68 IMMUNOREACTIVITY IN BRAINS OF NONHUMAN PRIMATES. L.C. Cork*, L.C. Walker, P. Davies§, and D.L. Price. (SPON: C.S. Duchala). Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182. §Depts. of Neuroscience and Pathology, Albert Einstein College of Med., Bronx, NY 10461.

A68, an antigen enriched in the brains of individuals with Alzheimer's disease (AD), is recognized by monoclonal antibody Alz-50 [Wolozin et al., Science 232:648, 1986]. In AD, A68 immunoreactivity occurs in neurons, including neurites in plaques and neurofibrillary tangles (NFT) in perikarya. Although trace amounts of A68 immunoreactivity may occur in some control human brains, antigens in these brains may be cross-reactive proteins that differ from A68 [Wolozin & Davies, Ann. Neurol. 22:521, 1987]. To determine whether A68 appears in brains of aged nonhuman primates (which develop plaques), we used immunocytochemical methods to examine brains of New World monkeys, Old World monkeys, and great apes. In these animals, A68 immunoreactivity is present in some neurons, fibers, and abnormal neurites associated with plaques. The number of A68-immunostained elements in all nonhuman primate brains examined was significantly less than that seen in humans with AD. These findings reinforce the usefulness of nonhuman primates in the study of processes that occur in the brains of individuals with age-related degenerative diseases.

437.12

INDUCTION OF "ALZHEIMER" ANTIGENS IN CELLS FROM NORMAL SUBJECTS BY THE MITOCHONDRIAL UNCOUPLER CCCP. A.C.Baker,* L.-W. Ko,* and J.P. Blass. Dementia Research Service, Burke Rehabilitation Center, Cornell University Medical College, White Plains, NY 10605.

When grown under conditions designed to favor the expression of neuronal properties, skin cells from all of 18 Alzheimer subjects tested stained with polyclonal and/or monoclonal antibodies to paired helical filaments (PHF) and/or with the Alz-50 antibody; cells from none of the 18 carefully matched controls did so.

However, when grown in the presence of a 100 nM conc. of the mitochondrial uncoupler CCCP, control lines then stained for PHF (8 tested with polyclonal and 2 tested with monoclonal antibody). The control lines then also stained with the Alz-50 antibody (3 lines tested). CCCP was used because of published evidence that mitochondria are partially uncoupled in Alzheimer's disease brain biopsies.

Induction of a metabolic lesion comparable to that in Alzheimer brain is adequate to cause expression of typical Alzheimer antigens. This culture system appears to be convenient for studying the biology of PHF formation.

437.13

ALTERED CASEIN KINASE ACTIVITY IN ALZHEIMER BRAIN. D. Iimoto* and T. Saitoh. U.C.S.D., Sch. of Med., Neuroscience Dept., M-024, La Jolla, 92093.

Protein phosphorylation has been shown to play an important role in regulating cell functions. In Alzheimer's disease (AD), there are proteins which are abnormally phosphorylated. These include P60 (a Mr 60,000 protein), P86 (a major substrate of PK-C) and tau. Since tau is associated with AD specific neurofibrillary tangles, its overphosphorylation may have an important role in the AD pathogenesis. Therefore, there must be some kinase(s), and/or phosphatase(s) which are involved in this overphosphorylation. Since cAMP stimulates tau phosphorylation, we examined the cAMP dependent phosphorylation of tau. Using 10μM cAMP and exogenous tau, there was no difference in the kinase activity between the control and diseased samples. Furthermore, no difference was seen in tau phosphatase activities of AD and control samples in the presence of Mg^{2+}, Mn^{2+} or Ca^{2+}/calmodulin. Because we had some evidence which indicated that casein kinase might also phosphorylate tau, we studied the activity of casein kinase. Casein kinases I and II were activated with spermine (1mM) and casein kinase II was inhibited with heparin (150 units/ml). Normal cytosolic fractions contained more spermine activated kinase activity and heparin sensitive kinase activity than did the AD cytosolic fractions. This indicates that there is less casein kinase II activity in brains with AD. While the differences found are not likely to account for the excessive phosphorylation of tau in AD, altered casein kinase II activity in Alzheimer brain may be involved in the pathogenesis of disease.

437.14

NEOCORTICAL PARVALBUMIN-CONTAINING NEURONS ARE RESISTANT TO DEGENERATION IN ALZHEIMER'S DISEASE. J.H. Morrison, K. Cox*, P.R. Hof, M.R. Celio. Research Institute of Scripps Clinic, La Jolla, CA 92037, and Dept. of Anatomy, University of Kiel, FRG.

The regulation of intracellular calcium levels is critically important to neuronal viability. Therefore we investigated the distribution of the calcium-binding protein parvalbumin (PV) in human neocortex and its relationship to cellular vulnerability in Alzheimer's disease (AD). In normal human neocortex, PV-immunoreactivity was confined to the non-pyramidal cells which were present in layers II-VI, with the highest density in layers II-IV. A quantitative analysis of the superior frontal gyrus demonstrated that the mean density of labeled neurons in layers II-IV of 3 control brains was 154 ± 13 labeled cells/mm^2, whereas the mean density in 7 AD brains was 166 ± 12. Therefore, PV appears to be a marker for a subset of neurons that are resistant to pathology in AD. A second calcium-binding protein, calbindin (CaBP), labels a different subset of non-pyramidal neurons that is largely confined to layer II. In addition, pyramidal neurons in layers III and V are lightly CaBP-immunoreactive. Preliminary analysis suggests that the heavily labeled neurons are resistant to degeneration in AD, whereas the lightly labeled neurons are vulnerable. Thus, calcium-binding proteins represent interesting new markers which distinguish between neurons that are susceptible to pathology in AD from those that are resistant.

437.15

QUANTITATIVE ANALYSIS OF NON-PHOSPHORYLATED NEUROFILAMENT PROTEIN (NPNFP)-IMMUNOREACTIVE NEURONS IN NORMAL AND ALZHEIMER'S DISEASE BRAIN. P.R. Hof, K. Cox*, and J.H. Morrison. Research Institute of Scripps Clinic, BCR-1, La Jolla, CA 92037, USA.

We have postulated that pyramidal neurons that are prone to neurofibrillary tangle (NFT) formation in Alzheimer's disease (AD) contain very high somatic and dendritic levels of NPNFP in the healthy aged cortex. In addition, the intracellular concentration of NPNFP is directly correlated with the cell size. In order to further investigate the relationships between cell size, intracellular concentration of NPNFP, and NFT formation, we performed a quantitative analysis of the distribution and density of NPNFP-immunoreactive neurons in normal and AD neocortex. In the AD brains, these distribution patterns were correlated with those of plaques and NFT. The immunoreactive cells were divided into three size groups : < 250 μm^2; 250-350 μm^2; > 350 μm^2. In layer III, a significant (72 %) loss was observed only in cells > 350 μm^2, whereas in layer V, there was a moderate decrease in the intermediate-sized cells (44 %), and a dramatic loss in the large cell group (78 %). Even though cell loss was only apparent among the larger neurons, a decrease in the intracellular concentration of NPNFP (as measured by optical density) was observed in all the three size groups. In all the AD cases, there was a high number of plaques and NFT in layers III and V. These data further support the hypothesis that a chemically-identified subset of pyramidal neurons is particularly vulnerable in AD. Supported by grants from ADRDA, AG O6647 and FNRS 83.495.0.87.

437.16

SERIAL SECTION COMPARISON OF TAU HISTOCHEMISTRY WITH A HIGHLY SENSITIVE SILVER IMPREGNATION METHOD FOR SENILE PLAQUES AND NEUROFIBRILLARY TANGLES. B.J.Quigley Jr.* K.S. Kosik and N.W. Kowall. (SPON: Bruce Ekstein). Neurology Service, Mass. General Hospital, Boston MA 02114.

We compared tau immunoreactive features to argyrophilic features using the sensitive Gallyas intensified Hick's procedure developed by Campbell et al (Soc Neurosci Abs 13:678) in the CA 1 field of the hippocampal formation of 5 patients with Alzheimer's disease (AD).

Tau histochemistry showed a plexus of dystrophic neurites (DN) spanning CA 1, with greatest density near the stratum oriens and radiatum borders. A band of typical neurofibrillary tangles (NFT) and occasional senile plaques (SP) were seen in the center of CA1.

In contrast, the silver method marked NFT throughout CA1. These included typical NFT seen with tau histochemistry and a group of larger diffusely argyrophilic tangles. DN were homogeneous in distribution and longer than tau reactive DN. SP were more dense and formed a continuous band in stratum radiatum. In one case showing early changes of Alzheimer's disease, the silver method showed marked homogeneously distributed SP only whereas tau histochemistry marked occasional DN and NFT. This may reflect tau protein's predisposition to mark an earlier stage of NFT formation in A.D.

437.17

A COMPUTER METHOD FOR 3-D RECONSTRUCTION OF THE LAMINAR AND REGIONAL DISTRIBUTIONS OF PLAQUES AND TANGLES IN ALZHEIMER BRAINS. C-N Kim*, C. White III*, D. Sparkman, D. J. Woodward and M-C de Lacoste.(SPON: G. Mihailoff) Depts. of Cell Bio. & and Path., U. T. Southwestern Medical Ctr., Dallas, TX 75235

A number of previous investigators have examined the laminar and regional distributions of neurofibrillary tangles [NFT] and neuritic plaques [NP] using small blocks of tissue sampled across different regions of Alzheimer [AD] brain [e.g., *Lewis* et al., 1987]. While these studies have provided critical quantitative information, it has been difficult to conclusively determine if the spread of AD pathology in the cerebral cortex [CC] follows orthograde, retrograde or both ortho- and retrograde routes. Our aim has been to develop computer-assisted methods 1) to obtain precise 3-D reconstructions of laminar and regional distributions of NP and NFT throughout the entire extent of temporal and occipital cortices within cyto- and myeloarchitecturally defined regions and 2) to superimpose the reconstructions on a 3-D visualization of the detailed connectivity patterns for these same regions. We believe that these methods will enable us to trace the spatial course of CC degenerative changes in AD.

Formalin-fixed blocks of tissue [up to 3X4 in] were cut at 50μm with a large-stage freezing microtome. Groups of 5 sections at 1mm intervals were processed using 1) Thioflavin-S, and PHF immunocytochemistry for NP and NFT labelling and 2) cresyl violet and the Gallyas silver stain for cyto- and myeloarchitecture. CSCAN and CARP 3D were utilised for computer-assisted regional differentiations, volumetric computations, NP and NFT counting and 3-D reconstructions [*Smith, de Lacoste*, et al 1985, 1987]. These methods are proving to be effective in delineating the relationship between the distribution of AD pathology and intracortical connectivity.

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437.18

MORPHOLOGIC CHANGES IN AMYGDALOID SUBREGIONS IN ALZHEIMER'S DISEASE. S.A. Scott, D.L. Sparks, C.A. Knox*, S.T. DeKosky and S.W. Scheff. Depts. Anatomy & Neurobiology and Neurology, Lexington VAMC and Sanders-Brown Research Center on Aging, University of Kentucky, Lexington, KY 40536.

The emerging picture of Alzheimer's Disease (AD) as disruption of specific cortical and subcortical structures necessitates closer examination of subregions of primary limbic structures affected by the disease. Since the amygdala is a principle limbic structure heavily involved in both the behavioral and pathological manifestations, we studied various amygdaloid subnuclei for differential alterations in AD. Three dimensional reconstructions of the entire amygdaloid complex were employed. Frozen 50 µm thick sections were obtained throughout the entire amygdala with every fifth section stained with cresyl violet.

The amygdala in subjects with AD demonstrates a striking topographical rearrangement and reduction in size. In AD the structure appears to have lost considerable volume, with secondary dilation of the inferior horn of the lateral ventricle (*ex vacuo*). The rostrocaudal extent of most nuclei, as well as the entire complex, is reduced. The nuclei reveal a distorted outline in relation to each other and to adjacent structures, most marked in the basolateral group. Volumetric changes in specific areas and nuclear groups will be discussed.
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437.19

HLA-DR-POSITIVE MICROGLIA IN NORMAL AND DISEASED BRAINS. L.A. Mattiace, G. Gong* and D.W. Dickson*. Dept. Pathol., Albert Einstein College of Medicine, Bronx, NY 10461.

Several recent studies using monoclonal antibodies specific to HLA-DR, a class II major histocompatibility cell surface glycoprotein, have demonstrated HLA-DR on a small number of microglia in the normal central nervous system. Far more HLA-DR-positive microglia have been described in Alzheimer's disease (AD), raising the possibility that immune mechanisms may be operative in AD. Brains were obtained at autopsies of 2.5 to 24 hours post-mortem from 14 individuals: 1 child, 2 young adults, 2 adults with neurologic disease, and 9 cases of AD (3 early, 6 chronic). Staining of microglia with antibodies to HLA-DR was highly sensitive to duration and type of fixation. Optimal staining was obtained with short duration PLP fixation, cyropreservation with sucrose and vibratome sections. HLA-DR-positive microglia were seen in both gray and white matter of all cases. With the exception of chronic AD, microglia with delicate, highly branched processes were uniformly distributed. In chronic AD, microglia with short, stubby processes or rounded contours were clustered. Double-labeling with glial fibrillary acidic protein demonstrated that HLA-DR-positive cells were not astrocytes. With electron microscopy, labeled cells were consistent with microglia. In AD, they had prominent phagolysosomes. Double-staining with antibodies to beta-amyloid synthetic peptides showed microglia associated with amyloid deposits.

437.20

THE DISTRIBUTION AND DENSITY OF ALZHEIMER-LIKE NEUROPATHOLOGICAL MARKERS IN NON-DEMENTED AND MILDLY DEMENTED BRAINS. J.L. Price, D.L. White* and P. Davis* (SPON: W.T. Thach), Depts. of Anatomy/Neurobiology & Psychiatry, Washington Univ. School of Medicine, St. Louis, MO 63110.

As part of an ongoing study, tangles, plaques and Alz-50 immunoreactive cells (A50ic) have been mapped in the brains of cognitively unimpaired and demented subjects, 59 to 87 years in age. The cognitive status of each case was assessed by premortem testing in the Memory and Aging Project of Washington Univ., or by a structured retrospective interview with a close relative. Serial sections through the ventral forebrain were stained with the Bielschowsky silver method, or immunohistochemically with the Alz-50 antibody, and then mapped and counted with the aid of a computerized microscope digitizer. To date 7 unimpaired cases, 3 very mildly demented cases and 4 severely demented cases have been thoroughly studied.

In the unimpaired cases, the highest density of tangles and A50ic was found in the perirhinal cortex, followed (in order) by the entorhinal cortex, anterior olfactory nucleus, and hippocampal field CA1. A similar pattern is found in the mildly demented cases, although the overall density of these markers is greater. The pattern also appeared to be continued in the severely demented cases, but the high density of tangles and A50ic in many other brain structures obscured their differential distribution. These results support the concept that there is a continuum between healthy aging and Alzheimer's Disease.

However, the incidence and distribution of plaques is more selective. Almost no plaques were found in the unimpaired cases, except for one case which also had a high density of tangles, while all but one of the demented cases had a high density of plaques.

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437.21

A Case of Non-Demented Alzheimer's Disease and Hippocampal Sclerosis With Normal Cholinergic Parameters in Basal Forebrain, Neocortex, and Hippocampus. R. Zweig,* K. Schegg,* J. Peacock, and D. Melarkey* (SPON: C. Ort). Reno VA Medical Center and University of Nevada School of Medicine, Reno, NV 89520.

Maximum senile plaque (SP) density >15/mm^2 in neocortex meets the pathological criterion for Alzheimer's disease (AD), but some non-demented individuals have both high SP counts and preserved choline acetyltransferase (ChAT) in neocortex. We confirm these results for a 76 year old non-demented man and extend previous work by analysis of acetylcholinesterase (AChE) isoforms. Microscopic exam showed abundant neocortical SPs, occasionally >15/mm^2, and rare neurofibrillary tangles (NFTs) in frontal, parietal, and temporal cortices. Amygdala had highest SP counts. Despite sclerosis of CA1 region in hippocampus, neither SPs, NFTs, nor granulovacuolar degeneration were found in hippocampal gyrus, although subiculum and parahippocampal gyrus had these abnormalities. No neuronal loss or NFTs were seen in basalis or septal nuclei. ChAT activity in hippocampus, septum, and parietal cortex was similar to 3 aged control and higher than 8 AD cases. Also, the tetrameric isoform of AChE (a marker of cholinergic innervation) was not reduced. Our results suggest that pathological alterations of the basal forebrain cholinergic system do not precede SP development. ChAT activity and AChE isoform patterns in hippocampus are unaffected by severe loss of CA1 pyramidal neurons.

437.22

DISCOVERY OF HYDROXYPROLINE AND AN ACTIN DETERMINANT IN THE INSOLUBLE PAIRED HELICAL FILAMENTS OF ALZHEIMER BRAIN. G. D. Vogelsang, G. E. Dean* and F. P. Zemlan. Department of Physiology and Biophysics, Department of Microbiology and Molecular Genetics, and The Alzheimer's Research Center, University of Cincinnati College of Medicine 45267.

Neurofibrillary tangles (NFT), comprised of paired helical filaments (PHF), are the critical neuropathological feature for the differential diagnosis of Alzheimer's disease (AD). Immunological studies indicate that several antigen-specific antibodies demonstrate cross-reactivity with "purified" PHF. It has not been shown, however, that this cross-reactivity is associated with the SDS insoluble PHF core protein (PHF_i) as opposed to PHF associated proteins or contaminants of the purification process. "Purified" PHF when subjected to electrophoresis (100V) in Tris/Borate/SDS buffer resulted in the separation of solubilized PHF associated proteins (PHF_s) from PHF_i. Electronmicroscopy revealed an intact PHF structure before and after this elutrapping technique (ET). A significant difference in several amino acids characterized PHF_i from PHF_s. The percent total mass of hydroxyproline (hpr) and glycine increased in the PHF_i after ET from 30 minutes to 4-1/2 hours. The elevation of these amino acids in PHF_i remained relatively constant from 1.5 to 18 hours after ET, peaking at 22% of the total protein after 39 hours. ELISA data confirmed that our PHF_i and PHF_s fractions were reactive with several putative PHF-specific antibodies (Ab's). Cross-reactivity of PHF_i with Ab's to other cellular components which include: microtubule associated protein-tau, ubiquitin and a newly identified determinant-actin, was demonstrated. None of the purported components of PHF contain hpr. Therefore, these data suggest that inappropriate hydroxylation of proline residues in PHF protein occurs in NFT. This hydroxylation may be the catalyst for the polymerization and subsequent insolubility of PHF in brain regions affected with AD.

437.23

NEUROFILAMENT GENES AND ALZHEIMER'S DISEASE: IS THERE AN ASSOCIATION? G. Lacoste-Royal*, M. Mathieu*, J. Nalbantoglu*, M. Freire*, J.-P. Julien*, S. Gauthier and D. Gauvreau* (SPON: L. Descarries). INRS-Santé, Pointe-Claire, Canada, H9R 1G6; Institut du Cancer de Montréal, Montréal, Canada, H2L 4M1 and McGill Center for Studies in Age and Aging, Montréal, Canada, H3G 1A4.

The primary cause of Alzheimer disease (AD) remains unknown, although the implication of genetic factors has been suggested, based on autosomal dominant transmission of the disease in several pedigrees. Neurofibrillary tangles, one of the pathological lesions of AD, possess epitopes of neurofilament (NF) proteins. In order to detect a possible anomaly that might relate to the disease, we searched for an association between the NF-L gene and AD in a group of sporadic AD cases. All cases met current clinical criteria for AD; in most cases the diagnosis was confirmed at autopsy. Genotypes for a TaqI RFLP at the NF-L locus (allele 1: 3.7 kb, allele 2: 2.9 kb) were determined for a control group and for our AD group. Allelic frequencies for allele 1 and allele 2 were respectively 0.72 and 0.28 for the control and 0.60 and 0.40 for the AD group. These differences were found to be not significant for the number of samples analysed. Thus our results showed no association between the gene for the 68 kd NF protein and AD. Supported by MRC Canada grant #MA-10027.

437.24

COMPARISON OF GROWTH-ASSOCIATED PROTEIN (GAP-43) FROM RAT AND HUMAN CORTEX, AND CHANGES IN GAP-43 IN ALZHEIMER'S DISEASE. J.K. Glenn* and P.D. Coleman. Department of Neurobiology and Anatomy. University of Rochester School of Medicine and Dentistry. Rochester, NY 14642.

Using SDS polyacrylamide gel electrophoresis and western blotting we have compared GAP-43 from rat and human cortex, and examined levels of GAP-43 in normal and Alzheimer's Disease (AD) hippocampus. Gap-43 was identified in western blots using a mouse monoclonal antibody (91E12) directed against rat GAP-43 (antibody provided by J.H.P. Skene, Stanford University.) followed by blot treatment with biotinylated horse anti-mouse IgG, avidin-linked horseradish peroxidase, and peroxidase substrate.

GAP-43 was detected both in the membrane-bound and cytosolic fractions of cell extract. Triton X-100 extractions of the membrane were able to remove some GAP-43, but complete extraction required dissolving the membranes in boiling SDS. We find that the rat and human GAP-43 show the same molecular weight.

In order to determine if post-mortem delay leads to changes in GAP-43, we have examined rat brain subjected to a variety of post-mortem conditions. Our data suggest that a broad range of post-mortem delay has little effect on amount or electrophoretic pattern of GAP-43.

In paired of AD and control cases matched for age and post-mortem delay, we find lower levels of GAP-43 in SDS extracts from AD hippocampi.

When GAP-43 was analyzed from SDS gels containing urea, we observed an increase in the heterogeneity of the protein. In these gels the GAP-43 from human tissue appeared to be more heterogeneous than the rat GAP-43.

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437.25

CHARACTARIZATION OF THE HUMAN GAP-43 GENE AND STUDIES OF mRNA LEVELS IN ALZHEIMER'S DISEASE. K.E. Rogers* A.B. Wadhams* N. A. Lebda* P.D. Coleman. (SPON: B. Weiss).Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY, 14642.

GAP-43 is a phosphoprotein which appears to be a marker of the development, growth, and regeneration of neuronal processes. A clone containing the human GAP-43 gene was isolated from a Charon 4A genomic library provided by T. Maniatis, using a 1.4 Kb rat GAP-43 cDNA probe (pGAP) previously charactarized by P. Skene and associates. Approximately 9 x 10^5 plaques were screened with the pGAP probe and a single positivily hybridizing clone was purified through three rounds of screening. The small number of positive plaques observed from a screen of 10^5 colonies suggests a low gene copy number, while the stringency of washing subsequent to hybridization was indicitive of a high degree of homology between the rat and human coding regions. The size of the insert is 17 Kb and is consistent with the manner in which the library was constructed. Restriction mapping and sequence data for the insert will be presented. We are currently identifying non-coding regions of the gene thought to be involved the transcriptional regulation.

Studies using radioactively labelled pGAP to probe total RNA from various brain regions indicate that the levels of message for GAP-43 are similar in Alzheimer's disease (AD) and control brain hippocampus. This finding contrasts with the approximate 35-45% excess loss of neurons in AD over the loss seen in normal aging.

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437.26

HEAT SHOCK PROTEINS AND UBIQUITIN IN CULTURED NERVOUS TISSUE

A.Morandi*, W.Welch, G.Perry, L.Autilio-Gambetti and P.Gambetti (SPON: P.Gambetti). Div. of Neuropathology, Case Western Reserve University, Cleveland OH 44106 and Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

We have used an in vitro system to investigate the response of the nervous tissue to stress conditions. Explants of rat dorsal root ganglia were exposed to two different types of stress conditions: aluminum intoxication and heat shock. Expression and cellular location of Heat Shock Proteins (HSPs) were analyzed on fluorograms and immunoblots after separation by one and two dimensional gel electrophoresis, and by immunocytochemistry using mono and polyclonal antibodies. Ubiquitin (Ub) conjugates were also analyzed. Both stress conditions resulted in an increase of the Ub-conjugates, with an higher increment in chronic aluminum intoxication. On the contrary, HSPs were detected only after heat shock treatment. These findings are consistent with the observation that intracytoplasmic inclusions induced by chronic administration of antimitotic drugs, which as aluminum act on cytoskeleton, react with Ub antibodies but not with antibodies to HSPs (Gambetti et al. Neuroscience 1988). However further studies are needed to determine whether the Ub and the heat shock systems can be triggered indipendently by different tipes of stress conditions. These observations may be relevant to the recent discovery that Ub is present in neuronal inclusions of Alzheimer's and other degenerative diseases, where, on the contrary HSPs have not been detected.(Supported by NIH Grants NS14503 and AG00795).

438.1

EFFECTS OF CARBACHOL and GALLAMINE ON WHOLE-CELL CURRENTS IN NEUROBLASTOMA-GLIOMA N1E-115 CELLS. D.O. Keyser, D. Doerner, and B.E. Alger, Dept. Physiol., Univ. of Maryland Sch. Med., Baltimore., MD 21201

The actions of carbachol (CCh) and gallamine were studied in mouse neuroblastoma-glioma N1E-115 cells under whole-cell voltage-clamp. Using K-filled electrodes, brief pressure-ejections of CCh (0.05-1 mM) from blunt pipettes elicited a transient inward current followed by a longer-lasting outward current. Gallamine (100-200 μM), a nicotinic and putative muscarinic M2 receptor antagonist, had no effect on resting membrane current but blocked the CCh-induced inward current without altering the outward phase. Atropine reduced the outward component, but did not affect the inward transient.

Bath application of CCh reversibly suppressed voltage-dependent K current and decreased membrane conductance. Using Cs-filled electrodes and saline containing Ba and TTX, CCh application reversibly reduced peak inward I_{Ba} elicited by depolarizing clamp steps from hyperpolarized holding potentials. This effect was inhibited by atropine but unaffected following bath application of gallamine. Characteristics of the I_{Ba} I/V relationship were unaffected by CCh.

These data suggest that nicotinic receptor activation initiates an inward current while muscarinic responses involve both activation of an outward current and modulation of voltage-gated channels.

438.2

TWO MEMBRANE CONDUCTANCE RESPONSES OF DISSOCIATED RAT SYMPATHETIC NEURONS TO MUSCARINE ARE COUPLED BY A PTX-INSENSITIVE GTP-BINDING PROTEIN. N.V. Marrion*, T.G. Smart* and D.A. Brown, MRC Neuropharmacology Research Group, Department of Pharmacology, London University School of Pharmacy, London, WC1N1AX, U.K.

Dissociated adult rat superior cervical sympathetic neurones (Marrion, N.V., Smart, T.G. & Brown, D.A. Neurosci. Lett.,77:55,1987) were whole-cell voltage-clamped using a single-electrode discontinuous amplifier. The patch pipette solution contained (mM): K aspartate, 150; KCl, 30; $MgCl_2$, 1; Na_2ATP, 1; EGTA, 0.5; HEPES, 5; NaOH, 3. (pH 6.7). This solution preserves the voltage-dependent K-current, I_M(Marrion, N.V., J.Physiol.,396:87P,1987). Cells were clamped at -30 to-45 mV and 1 sec hyperpolarizing commands applied to deactivate I_M.

Bath-application of dl-muscarine (1-10 uM) produced a reversible inward current accompanied by inhibition of I_M, as in intact adult ganglia (Constanti ,A. and Brown, D.A., Neurosci. Lett.,24:289,1981). In a proportion of cells an additional component of inward current due to an increase in a time-and voltage-insensitive conductance, reversing near 0 mV, also occured. Inclusion of the non-hydrolysable GTP-analogs, Gpp (NH)p (200 uM; n=4) or GTP- -S (500 uM; n=8) in the pipette solution mimicked both effects of muscarine. Effects of GTP-analogs were slow, requiring about 20 min to reach equilibrium. Both responses to muscarine persisted in neurons preincubated for up to 27 hr with 500 ng/ml Pertussis toxin (PTx).

We conclude that both I_M inhibition and increased conductance produced by muscarine involve activation of a PTx-insensitive GTP-binding protein.

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438.3

CHOLINERGIC SUPPRESSION OF BOTH ENDOGENOUS AND ISOPROTERENOL-INDUCED M-CURRENT IN ISOLATED SMOOTH MUSCLE CELLS IS MIMICKED BY A DIACYLGLYCEROL ANALOG. Lucie H. Clapp*, Stephen M. Sims*, Joshua J. Singer, and John V. Walsh, Jr. Dept. of Physiology, U. of Mass. Med. Sch., Worcester, MA 01655

The role of the second messenger diacylglycerol [DAG] in mediating muscarinic suppression of M-current, a type of K^+ current, in gastric smooth muscle cells isolated from Bufo marinus was examined using single microelectrode voltage-clamp techniques. Extracellular application of 60 μM sn-1,2-dioctanoylglycerol [diC_8], a synthetic DAG analog, reversibly suppressed endogenous M-current in the same way as acetylcholine [ACh], causing a net inward current associated with a conductance decrease. Current relaxations which occur in response to hyperpolarizing pulses and represent the voltage-dependent turn-off of M-current were also decreased by diC_8. The suppression of M-current by diC_8 was not always as complete as it was in the case of ACh. Like ACh, diC_8, also suppressed M-current induced by isoproterenol through the mediation of cyclic AMP (Sims et al. Science 239: 190, 1988). Thus, the dual regulation of M-current by isoproterenol and ACh appears to involve two second messenger systems. Supported by NIH DK31620 and NSF DCB8511674

438.4

MUSCARINIC RECEPTORS MAY BECOME UNCOUPLED FROM ION CHANNELS IN DISSOCIATED CELL CULTURE. J. E. Freschi, Dept. of Neurology, Emory Univ. Sch. of Med, Atlanta, GA 30322.

We have found that neurons dissociated from neonatal rat superior cervical ganglia frequently lack electrophysiological responsiveness to muscarinic agonists. We have undertaken studies to determine how these neurons lose their responsiveness and how expression may be regulated. We grew neurons in F12 medium supplemented with 10% fetal bovine serum and nerve growth factor (50 ng/ml). We co-cultured some neurons with previously plated confluent brain glia, and we treated some cultures with conditioned medium from rat heart cells in culture. We used the whole cell patch technique to measure membrane potential and to voltage-clamp neurons in the single electrode switched clamp mode (Axoclamp-2). Neurons that were unresponsive to muscarine (1 uM to 1 mM) expressed voltage-sensitive K^+ currents resembling m-current (similar kinetics, voltage sensitivity, and block by barium). To examine whether the neurons expressed muscarinic receptors we measured ^{3}H-QNB binding. The cultures bound an average of 388 fmoles/mg protein (n=4). In the unresponsive neurons, phorbol esters, dibutyryl cAMP, dibutryl cGMP applied externally, and changing the GTP, ATP or Mg^{2+} levels in internal dialysis solutions did not cause an electrophysiological response or increase the responsiveness of the neurons to muscarine. Neither growing the sympathetic neurons on a layer of central glia nor exposing them to conditioned medium from heart cultures affected their sensitivity to muscarine. We suggest that certain undefined tissue culture conditions cause the muscarinic acetylcholine receptor to be uncoupled from potassium ion channels, perhaps due to the failure of expression of ntracellular messenger. This may be similar to the loss of carbachol sensitivity in cultured chick ventricular heart cells reported by Siegel and Fischbach (Devel. Biol. 101:346, 1984).

438.5

ARACHIDONIC ACID INDUCES A POTASSIUM CURRENT IN ISOLATED GASTRIC SMOOTH MUSCLE CELLS. Richard W. Ordway*, John V. Walsh, Jr., and Joshua J. Singer. Dept. of Physiology, U. of Mass. Med. Sch., Worcester, MA 01655.

Responses to externally applied arachidonic acid (AA) were measured in isolated gastric smooth muscle cells from the toad, Bufo Marinus, using patch-clamp techniques. AA was dispersed directly into aqueous solutions and delivered by either pressure ejection from a pipette or bath superfusion.

In current clamp, AA (40 μM) caused a hyperpolarization associated with a large conductance increase. Under whole-cell voltage clamp, AA (5-40 μM) induced a current as large as several nA which reversed near the calculated E_K at three different external K^+ concentrations. This current showed some outward rectification but was clearly present at very negative potentials (-100 mV). Two K^+ channels (20-25 pS and 8-10 pS in 20 mM $[K^+]_{out}$, 130 mM $[K^+]_{in}$) appear to underlie the whole-cell current. Consistent with these findings, AA was found to block acetylcholine-induced contractions in the same cells.

Our initial results indicate that this AA response can be mimicked by fatty acids which do not enter eicosanoid pathways. Thus, the mechanism of action may differ from that for "S" K^+ channel activation in Aplysia neurons (Piomelli, et al., Nature 328:38, 1987). Supported by NSF grant DCB8511674 and NIH grant DK31620.

438.6

SUBSTANCES THAT AFFECT ARACHIDONIC ACID TURNOVER IN *APLYSIA CALIFORNICA* NERVOUS SYSTEM ALSO ALTER THE INWARDLY RECTIFYING POTASSIUM CURRENT OF GIANT NEURONS. R. Q. Carlson* and I. B. Levitan, Grad. Dept. of Biochem., Brandeis University, Waltham MA 02254.

Substances, known to affect arachidonic acid (AA) metabolism in *Aplysia* nervous system, have been found to alter the magnitude of an inwardly rectifying potassium conductance (I_R) in the homologous *Aplysia* giant neurons, R2 and LP1. I_R was studied in the giant neurons using two electrode voltage clamp. Inward rectification negative to the potassium equilibrium potential, and block by 500 μM $BaCl_2$, were used to identify I_R. The phorbol ester TPA decreased I_R about 50% at 500 nM, a concentration known to maximally stimulate AA turnover from lipid storage in *Aplysia* ganglia. Para-bromophenacyl bromide (BPB), an irreversible inactivator of phospholipase A_2, increased I_R several fold irreversibly. Indomethacin at saturating dose (250 μM) increased I_R about six fold. At 50 μM, indomethacin inhibited AA turnover in ganglia.

Serotonin (5HT) has previously been shown to increase I_R in several *Aplysia* neurons through stimulation of intracellular cAMP. A saturating dose of indomethacin did not interfere with the 5HT induced increase in I_R. Also, a saturating dose of 5HT (50 μM), or forskolin and IBMX at concentrations sufficient to mimic and occlude the effect of 5HT, did not block the response of I_R to indomethacin or BPB. We conclude, therefore, the effect of BPB or indomethacin is independent of the cAMP mediated increase in I_R.

We have previously observed constitutive turnover of AA from phospholipid storage in ganglia. We propose that this turnover provides a constant supply of AA responsible for inhibiting a majority of the I_R in giant neurons. Involvement of cAMP in this inhibition seems unlikely. However, the precise molecular mechanism of AA modulation of I_R remains to be determined.

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438.7

SEROTONIN DIFFERENTIALLY MODULATES TWO K^+-CURRENTS IN THE LEECH RETZIUS CELL. A.L. Kleinhaus, C.L. Sahley & J. Acosta-Urquidi, Depts. Neurol.& Bio., Yale Univ. New Haven, CT.

Bath application of 5-HT results in an increase in the interspike interval of the serotonergic Retzius (R) cell (Leake, Comp.Gen.Physiol.83:1986). In other preparations 5-HT modulates several ionic conductances. Yet, little is known about neuromodulatory mechanisms in the leech.

Therefore, we examined the effects of 5-HT on the duration of the Ca^{2+}-dependent action potential in the leech R cell. We found that 5-HT (20-100 μM) reduced the duration by about 55%, n=10, $p<.01$, suggesting that the drug acted on the underlying ionic conductances.

Using standard two-electrode voltage-clamp methods, we separated individual outward currents as previously described (Johansen & Kleinhaus, J.Neurophys.56:1986). The results show that 10 min after exposure to 5-HT I_A increased, n=9, avg 108% +/- 37(SEM), $p<.001$. No simultaneous change in inactivation kinetics occurred, $p>.1$. In contrast, I_K decreased, n=10, avg 44% +/-6(SEM), $p<.01$. In 20% of the preparations leakage slope conductance decreased, avg 32% +/- 3(SEM), $p<.02$.

Since I_A may play a role in the control of firing frequency, the results provide a plausible explanation for the increase in interspike interval seen in the R cell following exposure to 5-HT. This modulation could be one of the self-regulatory mechanisms by which the R cell titrates the amount of transmitter it releases.

438.8

NORADRENALINE (NA) INHIBITS A TRANSIENT OUTWARD CURRENT IN RAT SUPRAOPTIC (SON) NEURONS. C.W. Bourque, McGill Univ. Centre for Research in Neuroscience, Montreal, Canada, H3G 1A4

Alpha-1 adrenergic agonists depolarize rat SON neurons. This study was undertaken to characterize the ionic basis of this excitatory action.

Membrane currents were measured from 47 SON neurons in hypothalamic explants using a single electrode voltage clamp. From a holding potential of -65 mV, bath application of NA (0.5-50 μM) induced a small (<0.1 nA) but reversible inward current. This current displayed a bell-shaped voltage-dependence with a peak near -65 mV and could not be detected above -55 or below -75 mV. From a holding potential near -100 mV, NA (0.05-50 μM) also caused a dose-dependent reduction of the transient outward current (TOC) triggered by depolarizing commands. As previously reported (Bourque, 1988; J. Physiol. 397) this current is blocked by 4-Aminopyridine (4-AP) and shows an overlap of its activation and inactivation curves between ca. -75 and -55 mV. Using either voltage-clamp protocol, addition of 2 mM 4-AP could mimic and occlude the actions of NA measured in the same cell. These results strongly suggest that the depolarizing action of NA is mediated by the reduction of a steady-state "window" current carried through TOC channels.

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438.9

EFFECTS OF SOMATOSTATIN-14 AND SOMATOSTATIN-28 ON POTASSIUM CURRENTS IN RAT NEOCORTICAL NEURONS. H.L. Wang, C. Bogen*, T. Reisine, and M.A. Dichter. Depts. of Neurology and Pharmacology, Univ. of PA, and Graduate Hospital, Philadelphia, PA 19104.

Somatostatin-14 (SOM-14) is a 14-amino acid cyclic polypeptide which is found widely distributed throughout the central and peripheral nervous systems as well as in a variety of nonneuronal tissues. Originally isolated from hypothalamic extracts and found to inhibit secretion of growth hormone from pituitary cells, a more general role for SOM-14 as a neurotransmitter or neuromodulator has now been proposed.

Immunoreactive-SOM (IR-SOM) has been found to be particularly abundant in rat cerebral cortical neurons, both in whole brain sections and in dispersed cortical cell cultures. In addition, cultured cortical neurons secrete IR-SOM-like material into media and have high affinity surface membrane receptors for SOM.

In the present study, SOM-14 and SOM-28 (100nM) were applied to cultured rat neorcortical neurons (12-28 days in culture) with a pressure perfusion apparatus, and responses were examined using whole cell voltage clamp technique. Cells were bathed in physiologic HEPES buffered saline containing cobalt and TTX, and potassium currents were elicited by applying step depolarizations from a holding potential of -80 mV. 16 of 24 cells responded to SOM-14 perfusion with an increase in potassium currents, while 8 of 24 showed no response. Following perfusion with SOM-28, 14 of 17 cells had a decrease in potassium currents.

Complementary receptor binding assay on membranes of cultured neurons using [^{125}I]CGP 23996 demonstrated the presence of high affinity specific binding sites for SOM-14 (IC_{50} = 0.4 nM).

438.10

A PHOSPHATASE-RESISTANT ANALOG OF IP_3 ACTIVATES A POTASSIUM CONDUCTANCE IN RAT HIPPOCAMPAL PYRAMIDAL CELLS. Madeline McCarren, Barry V.L. Potter*†, and Richard J. Miller. Univ.of Chicago, Chicago, IL 60637 and †Leicester Univ., Leicester UK LE1 7RH.

Inositol (1,4,5) trisphosphate (IP_3) is believed to function as a Ca^{+2}-mobilizing second messenger in many cell types. However, the use of the naturally occurring form of IP_3 experimentally can produce ambiguous results since IP_3 may be degraded or converted to compounds with other actions. Therefore we used a phosphorothioate analog of IP_3 (DL-IPS_3), which is resistant to phosphatase degradation (BBRC 150:626-632, 1988), to investigate the actions of IP_3 in rat hippocampal pyramidal cells using standard electrophysiological techniques.

Cells impaled with electrodes containing 25-100mM IPS_3 required more current to fire action potentials than controls, although the action potential voltage threshold was unchanged. The current-voltage relationship showed strong delayed rectification in IPS_3 cells rather than the normal inward rectification. Moreover, even subthreshold depolarizations were followed by transient afterhyperpolarizations in IPS_3 cells. These effects were not due to turning off of an inward current, as they persisted in TTX or Ca^{+2}-free solutions. The IPS_3 response was also unaffected by a reduction in extracellular Cl^- but was reduced in elevated K^+ , suggesting involvement of a K^+ conductance.

438.11

K^+ CHANNELS EVOKED BY BRADYKININ AND INOSITOL-1,4,5-TRISPHOSPHATE IN NG108-15 NEURONAL CELLS. H. Higashida[1], K. Furuya[2] & D. A. Brown[3]. [1]Department of Biophysics, Kanazawa University, Kanazawa 920, Japan, [2]National Institute for Physiological Science, Okazaki 444, Japan & [3]Department of Pharmacology, University College London, London WC1E6BT, U. K.

Activation of phosphatidylinositide breakdown in neuroblastoma x glioma (NG108-15) hybrid cells by bradykinin (BK) results in the generation of an outward K^+ current through the release of Ca^{2+} by the intermediary messenger inositol-1,4,5-trisphosphate ($InsP_3$). Channels mediating this outward current were investigated using cell-attached patch electrodes. Intracellular iontophoretic injection of InsP3 or Ca^{2+} ions, or extracellular application of BK evoked bursts of K^+ channels coincident with cell hyperpolarization measured with an intracellular recording micropipette. The most frequent channels had a mean single channel conductance of about 38 pS in symmetrical K^+ solutions; additional openings of lower conductance (18 pS)channels were also detected. The opening probability of these channels was not clearly dependent on pipette potential. Bath application of phorbol dibutyrate (PDBu, 1 uM) increased the number and opening probability of the $InsP_3$-induced channels without changing unitary conductance. The effects of PDBu suggest a possible interaction of the two products of phospatidylinositide breakdown at the level of the Ca^{2+} -dependent K^+ -channel.

438.12

RELATIONSHIP BETWEEN CALCIUM LOAD AND THE DECAY RATE OF I_{AHP} IN BULLFROG GANGLION NEURONS. Peter S. Pennefather and Joanne W. Goh, Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 2S2.

Action potential activity in bullfrog ganglion cells induces a Ca activated K current (I_{AHP}) whose decay reflects clearance of a Ca load from the intracellular vicinity of I_{AHP} channels. We have varied the calcium load by using 2 or 50 ms current clamp steps to evoke either single action potentials or bursts of 3-4 action potentials. Trains of bursts were generated by providing 50 ms rest periods between bursts. At the end of the last current-clamp command a single electrode voltage-clamp was engaged to measure I_{AHP} and its decay. Decay rate was constant over most of the time course of the current. In 70% of cells however, distortions due to K-accumulation were apparent at early times (< 80 ms). Accordingly, decay rate was estimated only after 80 ms had elapsed and the initial amplitude of the current was estimated by extrapolation. A single burst was sufficient to produce a near maximal current. In 0.5 mM Ca, the decay rate of I_{AHP} following a single burst was 5.8 ± 0.5/s . Following a train of 10 bursts, the decay rate was 1.6 ± 0.2/s. There was a linear relation between the log of the decay rate and the log of the number of bursts (r = 0.98, slope = -0.54). A similar relation was observed in 5 mM Ca (r = 0.99; slope = -0.59) but, a third fewer bursts were needed to produce an I_{AHP} with a given decay rate; the decay rate was 3.8 ± 0.3/s following a single burst and 0.92 ± 0.07/s following a train of 10 bursts. The rate of removal of the Ca load was estimated by measuring the decay rate of I_{AHP} evoked by a single action potential and determining the degree of reduction of this parameter as a function the time from the end of a given loading procedure. In 4 mM Ca, the recovery rate was 1.01/s following a single burst and 0.40/s following a train of 20 bursts. The mean decay rates of I_{AHP} associated with the two loading procedures were 2.90/s and 0.81/s, respectively. The difference between decay rates and recovery rates suggests cooperativity in activation of the K channels by Ca. The decrease in decay rate of I_{AHP} with increasing Ca loads may be due to the presence of a rapidly equilibrating, low affinity, high capacity Ca buffer that slows diffusion of Ca away from K channels. Local saturation of such a buffer would generate intracellular domains of high free Ca levels, centered on Ca channels, that shrink only slowly after Ca channels close. In this model, the decay of I_{AHP} would reflect the mutual disposition of the Ca and K channels. Quercetin, replacement of Na by Li and caffeine had only minor effects on decay rate of I_{AHP}.

438.13

INTRACELLULAR INJECTION OF CaM KINASE II REDUCES SPIKE AFTER-HYPERPOLARIZATION IN CULTURED SPINAL CORD NEURONS. S. Sombati, R.R. Forman, B.L. Attema*, K.A. Tennes-Rees*, W.C. Taft* and R.J. DeLorenzo. Dept. Neurology, Medical College of Virginia-VCU, Richmond VA 23298.

Calcium-calmodulin-dependent protein kinase (CaM kinase II) has been shown to modulate specific K^+ and Ca^{2+} currents in invertebrate neurons (Sakakibara, et al., Biophys. J. 50:319, 1986). To investigate a possible role of this kinase in vertebrate neurons, we have intracellularly injected purified CaM kinase II into spinal cord (SC) neurons cultured from embryonic rats. Each spike in a cultured SC neuron is followed by a large (5-10 mV) and prolonged (30-50 ms) after-hyperpolarization (AHP). Injection of CaM kinase II resulted in a reduction in both amplitude and duration of AHP, and sometimes spike broadening (n=11 cells). CaM kinase II effects on AHP were observed 4 min post-injection and peaked after 5-6 min. In some neurons AHP was totally abolished. Neither vehicle nor heat-inactivated CaM kinase II injection (n=8 cells) produced these effects. Preliminary studies suggest AHP is enhanced by injection of CaM kinase II monoclonal antibody that inhibits CaM kinase II activity in vitro and labels SC neurons immunocytochemically. This observation suggests a role for endogenous CaM kinase II in modulating AHP. Characterization of AHP indicates that it is due predominantly to Ca^{2+}-dependent K^+ conductances (Zhang & Krnjevic, Neurosci. Lett. 74:58, 1986). These results indicate that CaM kinase II regulates AHP in SC neurons and are consistent with the hypothesis that the effects of CaM kinase II on K^+ and/or Ca^{2+} currents may be responsible for the effects of this kinase on AHP.

438.14

TRANSFORMED 3T3 CELLS EXHIBIT A CALCIUM-ACTIVATED K CURRENT WHICH IS ABSENT IN NONTRANSFORMED CELLS. **S.G. Rane & L.F. Fleischman*. Dept. of Physiol., Tufts U. Med. Sch., Boston, MA 02111.**

Mitogenically stimulated or oncogenically transformed cells have been reported to have augmented trans-plasma membrane cation fluxes. Using the whole cell patch clamp technique we now report that Balb 3T3 fibroblasts, stably transformed with the viral Kirsten ras oncogene, exhibit a Ca-activated K current which is either absent or unavailable for activation in their nontransformed (normal) counterparts.

3T3 cells were grown to confluence (DMEM, 10% CS), dispersed with trypsin, triturated in medium, and allowed to settle out on plastic dishes. Recording solution was (in mM): 138 NaCl, 1 CaCl , 6 KCl, 1 MgCl , 10 HEPES, and pipette solution was 100 KCl, 50 NaCl, 10 HEPES, 5 MgATP, 0.1 BAPTA, making reversal potentials for K, Cl, and Na, -70, 0, and 25 mV respectively. Cells were held at -70 mV and stepped to 0 mV for 500 msec at 1 Hz. Currents were activated either by 300 nM A23187 or by 100 nM bradykinin (BR), a Ca mobilizing peptide, applied by pressure ejection from blunt-tipped pipettes.

86% (24/28) of *ras* transformed 3T3 fibroblasts responded to A23187 with outward current at 0 mV (mean amplitude = 0.6 ± 0.11 nA) and 92% (12/13) gave identical responses to BR (1.09 ± 0.12 nA); however, only 13% (4/30) of normal fibroblasts responded to A23187 and none (0/6) responded to BR. Peak outward currents in two of the normal cells were equivalent to those in transformed cells while responses in the other two cells were only 20 and 40 pA. Both cell types responded to A23187 and BR with a very slowly activating, persistent inward current at -70 mV. This current was blocked by 5 mM BAPTA internal, suggesting that it is activated by elevation of internal Ca. This result argues against the possibility that normal cells were not being sufficiently loaded with Ca to activate the outward current. The outward current response in the transformed cells was also eliminated with 5 mM BAPTA in the pipette solution. Using voltage ramps the outward current was shown to reverse at the K reversal potential and it was fairly linear from -80 to 20 mV. This result and insensitivity to TEA block suggests that it is not the large conductance Ca-activated K current described in many other tissues. Experiments are underway in which transforming *ras* protein will be added to the patch pipette solution in an attempt to enable outward current responses in normal 3T3 cells.

438.15

ACTION POTENTIAL CHARACTERISTICS OF SEGMENTALLY DEMYELINATED OPTIC TRACT (OT) AXONS FOLLOWING POTASSIUM CHANNEL BLOCKERS. D. A. Fox, D.-Y. Ruan and Y. Blocker*. U. of Houston, College of Optometry, Houston, TX 77004.

The role of potassium channel blocking agents in altering conduction properties of demyelinated PNS axons has been examined, however, similar studies have not been conducted in CNS axons. Our in vivo studies examined the frequency-dependent effects of vehicle (baseline), 4-AP and 4-AP + TEA on compound action potential (CAP) characteristics and excitability properties in fast (t1) and middle conducting (t2) OT axons in two different models of demyelination, segmental and paranodal (see following abstract), after optic chiasm stimulation. Segmental demyelination, produced by developmental and continuous lead exposure, was confirmed in 90 day old hooded rats using electron microscopy. In t1 and t2, lead decreases conduction velocity and amplitude (AMP), and increases rise (RT) and fall time, duration (DUR), and chronaxie. In t2, lead increases absolute and relative refractory periods (RRP), decreases frequency following (FF) and produces a subnormality. Compared to baseline, 4-AP effects are generally larger in t2 than in t1 (e.g., 4-AP decreases AMP and FF, and increases DUR, FT, and RRP). TEA effects are similar to those produced by 4-AP, however, as stimulus frequency increases they are larger in t1 than in t2. Large and medium diameter axons exhibit differential frequency-dependent sensitivity to 4-AP and TEA following segmental demyelination. Supported by ES 03183 (DAF).

438.16

ACTION POTENTIAL CHARACTERISTICS OF PARANODALLY DEMYELINATED OPTIC TRACT (OT) AXONS FOLLOWING POTASSIUM CHANNEL BLOCKERS. D.-Y. Ruan, D. A. Fox and Y. Blocker*. U. of Houston, College of Optometry, Houston, TX 77004.

The role of potassium channel blocking agents in altering conduction properties of demyelinated PNS axons has been examined, however, similar studies have not been conducted in CNS axons. Our in vivo studies examined the frequency-dependent effects of 4-AP and 4-AP + TEA on compound action potential (CAP) characteristics and excitability properties in fast (t1) and middle conducting (t2) OT axons in two different models of demyelination, paranodal and segmental (see preceding abstract), after optic chiasm stimulation. Paranodal demyelination, produced by 2,5-hexanedione (2,5-HD) exposure, was confirmed in 5-7 month old hooded rats using electron microscopy. 2,5-HD decreases conduction velocity, amplitude, rheobase and frequency following and increases rise and fall time, duration, chronaxie and absolute refractory period in t1 and t2. T2 axons have supernormality and a correlated decrease in relative refractory period. The 4-AP effects are almost identical to those observed in lead-exposed rats with segmental demyelination (see preceding abstract): 4-AP effects are generally larger in t2 than in t1. In contrast, TEA effects are only observed at low stimulus frequencies and generally only in t2. Paranodally and segmentally demyelinated large and medium diameter OT axons have similar sensitivity to 4-AP and completely different sensitivity to TEA. Supported by ES 03183 (DAF).

438.17

A DIFFUSION MODEL OF $I_{Na,cAMP}$. R.-C. Huang and R. Gillette. Dept. Physiol. & Biophys. and Neural & Behav. Biol. Prog., Univ. Illinois, Urbana, IL 61801.

The cAMP-activated Na^+ current ($I_{Na,cAMP}$) in molluscan neurons is independent of phosphorylation and diffusion-limited in its kinetics. These characters permit precise calculation of cAMP diffusion coefficient and phosphodiesterase (PDE) kinetic parameters.

Use of a diffusion-limited model permits precise modelling of the $I_{Na,cAMP}$ response to pulsed intracellular injection of cAMP, including effects of relocating the point source of cAMP within the neuron soma and bath addition of PDE inhibitors. The apparent diffusion coefficient of cAMP is estimated from the relation of latency to peak response and cell radius. PDE activity is extracted as a first-order rate constant from the exponential decay of $I_{Na,cAMP}$, while Michaelis-Menten parameters (K_m, V_{max}) are obtained by curve-fitting the $I_{Na,cAMP}$ response to an explicit finite difference equation.

Justification of a diffusion-limitation assumption is provided by counter-examples of other cAMP-stimulated currents with slower kinetics.

438.18

CO-REGULATION OF $I_{Na,cAMP}$ BY cAMP AND Ca^{2+}: A COMPETITIVE BINDING MECHANISM. R. Gillette and R.-C. Huang. Dept. Physiol. & Biophys., Neural & Behav. Biol. Prog., U. of Ill., Urbana IL 61801.

Intracellular Ca^{2+} and cAMP interact to co-regulate $I_{Na,cAMP}$ in pedal ganglion neurons of the mollusk Pleurobranchaea. Activity- and voltage-induced increase in $[Ca^{2+}]_i$ suppresses the $I_{Na,cAMP}$ response to injected cAMP and confers voltage-dependence. In contrast, high levels of cAMP reduce voltage dependence and antagonize Ca^{2+}-dependent suppression of the $I_{Na,cAMP}$ response by depolarization.

Ca^{2+}-activation of phosphodiesterase in suppression of $I_{Na,cAMP}$ is not confirmed by response kinetics and pharmacology. Instead, experimental data are best fit by a unifying model of antagonistic allosteric effects of Ca^{2+} and cAMP in regulating channel activity. Bound Ca^{2+} suppresses, while cAMP stimulates, ion current. Binding of one ligand antagonizes binding of the other.

Data indicate one-to-one binding of each ligand. A competitive binding mechanism explains apparent voltage-dependence of cAMP dissociation, the IV curve for $I_{Na,cAMP}$, and antagonism of Ca^{2+} suppression effects by high levels of intracellular cAMP. Experimental results and theoretical predictions agree well.

439.1

AXON VS. TERMINAL: ANALYSIS OF STIMULATION PARAMETERS EXCITING SINGLE DORSAL ROOT FIBERS. N.C. Tkacs and R.D. Wurster. Department of Physiology, Loyola University Medical Center, Maywood, IL 60153.

The present study was designed to characterize differences in electrical stimulation responsiveness between axons and axon terminals. The experimental model is the bullfrog hemicord, in vitro . Single dorsal root fibers are isolated and action potentials recorded in response to dorsal root (DR) and dorsal horn (DH) stimulation.

The results reported here are based on experiments in which responses were elicited from DR only (n=5), from DH only (n=6) and from both DH and DR (n=2). Chronaxies to DH stimulation were significantly longer (mean = 167.5 usec, S.D. = 28.2) than to DR stimulation (mean = 108.3 usec, S.D. = 19.2) ($p<.05$, unpaired t-test). When chronaxie measurements could be made at both sites on a single fiber, DH values were almost twice DR values (Fiber 1: DR - 85 usec, DH - 160 usec; Fiber 2: DR - 110 usec, DH - 200 usec). These data may indicate a region of decreased safety factor for conduction as an action potential proceeds down an axon to the terminal region.

Further studies will use this model to evaluate the sensitivity of these two stimulation sites to agents blocking calcium and potassium channels.

439.2

SMALLER MEPP AMPLITUDE IN THE DISTAL VERSUS PROXIMAL REGION OF THE FROG NMJ USING SIMULTANEOUS INTRA AND EXTRACELLULAR RECORDING. L.P. Fortier* and J.P. Tremblay. (Spon.: L. Larochelle) Lab. of Neurobiology, Laval University, Quebec, Canada, G1J 1Z4

The present experiments were undertaken to confirm our previous observations that the MEPP amplitudes were smaller in the distal region of the frog neuromuscular junction (NMJ) than in the proximal region (near the point of contact between the axon and the muscle fiber). These results were obtained with the Spatial Decay Method using simultaneous intracellular recording with 2 electrodes (Tremblay et al. Brain Res. 328, 170, 1985). It was attributed to the presence of smaller postjunctional folds (PJFs) (Robitaille et al., Soc. Neurosci. Abstr., Vol. 13 Part 1, p. 317) and/or smaller active zones in the distal regions. To further investigate this finding, intracellular recordings were made in presence of 6-9 μM neostigmine with an electrode placed halfway between the distal end and the proximal region. During the intracellular recording period, an extracellular electrode was placed alternatively at a distal and at a proximal site. The temporal correspondence between the extracellular and intracellular signals permitted us to identify two MEPP populations originating from the distal and proximal regions. In the majority of these experiments we observed that the intracellularly recorded MEPPs originating from the distal region were significantly smaller than those produced in the proximal region of the same NMJ. This confirms Tremblay et al. observations using the Spatial Decay Method. A significant correlation was observed between the amplitude (in μV) and the area (in μV. ms) of the extracellularly recorded MEPP. In addition, for a given extracellular MEPP amplitude the area under the curve tends to be smaller for the signals coming from the distal regions. In the presence of anticholinesterase, the ACh could have rebound to multiple sites along the PJFs. Thus our observations suggest that ACh would rebind fewer times to ACh receptors in the distal region caracterized by shorter PJFs.

439.3

DIFFERENT CA^{++} SENSITIVITY OF SPONTANEOUS RELEASE ALONG THE FROG NEUROMUSCULAR JUNCTION. J.P. Tremblay and R. Robitaille. (Spon.: Radouco-Thomas, S.) Lab. of Neurobiology, Laval University, Quebec, Canada, G1J 1Z4.

Spontaneous transmitter release has been recently characterized along the frog neuromuscular junction (NMJ). Miniature endplate potentials (MEPPs) occur more frequently and are larger in proximal regions (near axon termination on muscle fibre) than in distal ones. The present experiments were done to investigate the possible roles or implications of Ca^{++} in these nonuniformity of spontaneous release. A new method (microperfusion technique) has been elaborated to apply a Ca^{++} solution at a limited portion of the nerve terminal (30 μm) and thus allowed us to study the effect of Ca^{++} on different regions of the nerve terminal. Experiments were performed on the frog cutaneous pectoris muscle placed in a 0mM Ca^{++} - 5mM Mg^{++} Ringer solution. NMJs were localized with a microscope equipped with Nomarski optics and MEPPs were recorded intracellularly. The intracellular electrode was placed at equal distance between the two regions to be microperfused with a Ca^{++} stream. This implicates that the spatial attenuation of MEPP amplitude towards the electrode is about the same and thus allows us to compare the amplitude of MEPPs produced in both regions. Another electrode (tip of 10 μm) filled with a solution of 5mM Ca^{++} and 40 mg/ml of Dextran Blue was used for the application of Ca^{++} in parallel with a high rate of bath perfusion which allows a restricted application. Several controls were performed to insure that the stain had no effects on the preparation and that the spread of the stained solution corresponds to the presence of Ca^{++}. The application of 5 mM Ca^{++} induced a larger increase of MEPP frequency which proceeds more rapidly in proximal regions than in distal ones. Moreover, MEPPs produced by the proximal regions are proportionally larger than those produced by the distal region. This confirms our previous observations using two intracellular electrodes. The present results indicate that nonuniformity of transmitter release can be related to Ca^{++}- dependant mechanisms. (Supported by MRC grant to JPT and MRC studentship to R.R.).

439.4

NONUNIFORM TRANSMITTER RELEASE EFFICACY ALONG THE FROG NEUROMUSCULAR JUNCTION R. Robitaille, and J.P. Tremblay. Lab. of Neurobiology, Laval University, Quebec, Canada, G1J 1Z4

The efficacy of the release sites has been reported to be different along the frog neuromuscular junction (NMJ). Endplate potentials (EPPs) are produced more frequently by the proximal regions than by the distal ones in conditions of reduced transmitter release probability (low extracellular Ca^{++}). Experiments discussed here deal with the efficacy of proximal and distal regions of the NMJ to evoked release in higher Ca^{++} concentrations using the microperfusion technique (see preceding abstract). Experiments were performed on the cutaneous pectoris nerve-muscle preparation placed in a 0mM Ca^{++} - 5mM Mg^{++} Ringer solution. EPPs were recorded intracellularly between the regions to be microperfused by the Ca^{++} solution. The Ca^{++} solution containing 2 mM Ca^{++} and 40 mg/ml of Blue Dextran was applied successively at the proximal and distal regions from a focal electrode (tip of 10 μm). During the microperfusion of Ca^{++}, pulse pairs (interval of 15 msec) were applied every two seconds by stimulating the nerve with a suction electrode. EPPs were subthreshold since only a reduced number of release sites participated in evoked release due to the restricted application of Ca^{++}. Quantal content was evaluated by dividing the mean amplitude of the first evoked response by the mean MEPP amplitude. EPPs evoked by the proximal regions had a larger quantal content (4.6 to 14.25, mean 8.94) than the distal regions (1.6 to 8.3, mean 5.07). In two occasions, it was impossible to evoke transmitter release in the distal region whereas the proximal regions evoked fairly large EPPs. Furthermore, failures to evoked release are less frequent in proximal regions (8%) in comparison to the distal ones (28%). The difference between EPP_2 and EPP_1 was significantly larger in proximal then in distal regions however the facilitation ($F=(EPP_2-EPP_1)/EPP_1$) was not different. All these results indicate that the proximal region of the frog NMJ is stronger and more efficient than the distal region. Supported by MRC grant (JPT) and MRC studentship (R.R.).

439.5

THE ABSENCE OF DETECTABLE NON-QUANTAL ACH RELEASE FROM ACTIVE ZONE REGIONS OF FROG NERVE TERMINALS. S.D. Meriney, S.H. Young, C.B. Gundersen, and A.D. Grinnell. Jerry Lewis Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024.

Non-quantal release of acetylcholine (ACh) from motor nerve terminals has been assumed to occur from active zone regions. However, the localization of this leakage has not been directly determined. We have used an outside-out patch of muscle membrane as a sensitive detector of ACh released from localized regions of enzymatically detached frog nerve terminals. Quantal events (EPCs and MEPCs) could be localized to 3-5 releasing sites at any one patch electrode position along the terminal. Using this method, we were unable to detect significant transmitter leakage from active zone regions of the nerve terminal. Biochemical measurements of ACh leakage did not reveal any significant difference between enzyme treated and control preparations. These results suggest that ACh leakage at adult frog terminals either is localized to a source other than the active zone region of the terminal, or is released diffusely from many sources, which may include the nerve terminal.
Supported by grants from the MDA, NIH, and NSF.

439.6

INHIBITORS OF VESICULAR ACETYLCHOLINE TRANSPORT AT MOUSE AND FROG NEUROMUSCULAR JUNCTIONS. S.P. Yu* and W. Van der Kloot. Dept. of Physiology & Biophysics, SUNY, Stony Brook, NY 11794.

Pretreatment in hypertonic solution increases MEPP, MEPC and uni-quantal EPP sizes roughly twofold. Responses to iontophoretic or bath applied ACh were unchanged by the pretreatment. The increases in quantal size were prevented by adding to the hypertonic solution inhibitors of active ACh transport into synaptic vesicles, like 1 μM AH5183. The inhibitors have little, short term effect on MEPP or MEPC size in untreated preparations. However, after hypertonic pretreatment, the inhibitors promptly decreased the size of mouse MEPPs and MEPCs about 50 %.

In the frog, the inhibitors had little effect except when the hypertonic solution was made with Na gluconate (replacing NaCl). Then MEPP and MEPC sizes increased about fourfold, and the inhibitors promptly decreased size about 50%. Apparently, these pretreatments add to the quantal release process a second component , which can be quickly blocked by the inhibitors. In the frog, this second component of release was also reduced by raising $[K^+]_{out}$, so it is questionable whether the additional release is by exocytosis.

439.7

UNIFYING THEORY FOR THE BASIS OF THE DIFFERENT CLASSES OF QUANTA FOUND IN THE NEUROMUSCULAR JUNCTION BASED ON SUB-UNITS. M.E. Kriebel, J. Vautrin and F. Llados. Dept. of Physiology, SUNY Health Science Ctr., Syracuse, NY 13210

There are three quantal classes normally found in the frog and mouse neuro-muscular junctions based on amplitude and different pharmacological effects. Amplitudes show bell and skew classes separated by a discontinuity in the distribution. Giant MEPPs form a skew distribution and are increased with various treatments. Day-old mice, re-innervated junctions, BTX, β-bungarotoxin poisoned junctions and those induced to release large numbers of MEPPs show mainly skew-MEPPs. Nerve stimulation releases bell-MEPP sized quanta. Integral peaks in the amplitude distributions show that both classes are composed of sub-units (sub-MEPP size) (Kriebel et al., 1982, J. Physiol.; Erxleben and Kriebel, 1988, J. Physiol.). We report slowly rising MEPPs of all 3 classes which are potentiated after high rates of release following heat, hypertonic saline and 4-amino-quinoline. Many MEPPs start with a sub-MEPP and have offsets on the rising phase. We propose that there is a counting and timing mechanism for the release of sub-units. Bell-MEPPs would be composed of 7-10 sub-units synchronously released whereas skew-MEPPs are composed of sub-units fitting a Poisson distribution. Giant MEPPs would represent two or more bell-MEPPs. Slow and irregular MEPPs of all classes result from a disruption in the sub-unit synchronization mechanism. N.S.F. 19694.

439.8

EVIDENCE FOR QUANTAL TRANSMITTER RELEASE AT A CNIDARIAN SYNAPSE. P.A.V. Anderson. Whitney Lab. and Depts. of Physiology and Neuroscience, Univ. of Florida, St. Augustine, Fl 32086.

Chemical synapses in cnidarians contain a few, large, heterogeneous, membrane-bound structures collectively termed synaptic vesicles. Given this heterogeneity of vesicle size and shape, various modes of transmitter release ranging from non-vesicular to quantal could be envisaged. Synapses in the motor nerve net of the jellyfish Cyanea were examined using patch pipettes for simultaneous intracellular recordings from the pre- and post-synaptic cells. Only cells forming a single, discrete, en passant synapse were used. Action potentials were generated (1Hz) in the pre-synaptic cell by current injection, and the EPSC recorded under voltage clamp from the post-synaptic terminal. EPSC amplitude was reduced with a high Mg^{++}, low Ca^{++} saline. EPSCs occurred in discrete, approximately 10pA size-classes. The run-down of quantal size (m) during the course of the recordings, made statistical analyses impossible, but the fact that EPSCs occurred in discrete amplitude steps strongly suggests that transmitter release at these synapses is quantal. (Supported by NSF Grant BNS 85-06193.)

439.9

BACLOFEN DECREASES SYNAPTIC INHIBITION IN CULTURED HIPPOCAMPAL NEURONS BY A PRESYNAPTIC MECHANISM THAT IS INSENSITIVE TO PERTUSSIS TOXIN

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I have studied the actions of (-)baclofen at inhibitory synapses between rat hippocampal neurons in culture. Simultaneous whole-cell patch recordings were made from pre- and post-synaptic neurons, using an intracellular solution based on 145mM K gluconate. Individual bicuculline-sensitive IPSPs were elicited by a single electrically evoked presynaptic action potential. (-)Baclofen (1-20μM) decreased the amplitude of evoked IPSPs; under voltage-clamp at -40mV, the outward-going evoked synaptic currents (IPSCs) were reduced as expected by (-)baclofen (IC_{50}~5μM); (+)baclofen was inactive. IPSCs were reduced at all potentials by (-)baclofen, without change in E_{IPSC}. (-)Baclofen did not reduce Cl^- current responses of postsynaptic neurons to GABA, nor did it increase somatic membrane conductance. The presumed presynaptic action of (-)baclofen was not significantly antagonized by phaclofen (0.2-0.5mM); nor was it prevented by pre-incubation of the tissue with pertussis toxin (0.1-1μg/ml, 12-24h., 34-37^{o}C). I conclude that the reduction of inhibition by (-)baclofen results from its action at a presynaptic $GABA_B$ 'autoreceptor' on the terminals of GABAergic inhibitory neurons. The mechanisms by which (-)baclofen reduces GABA release appear to differ from those mediating reduction of Ca^{2+} currents in sensory neurons (Holz et al., *Nature*, 319, 670-672, 1986) and activation of K^+ conductance in adult rat neurons (Andrade et al., *Science*, 234, 1261-1265, 1986), both pertussis toxin-sensitive processes.

439.10

AN INTRACELLULAR STUDY OF PRIMARY AFFERENT DEPOLARIZATION IN THE ISOLATED BULLFROG SPINAL CORD. Y. Peng and E. Frank Depts. of Physiology and Neurobiology, Anatamy and Cell Science, Univ.of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

The neural transmitter GABA has been shown to cause primary afferent depolarization (PAD). Recently, we have shown that activation of presynaptic $GABA_A$ as well as $GABA_B$ receptors causes presynaptic inhibition of synaptic transmission between muscle spindle afferents and spinal motoneurons in the isolated bullfrog spinal cord. To demonstrate that $GABA_A$ receptors also mediate PAD, we studied the pharmocology of synaptic potentials evoked by single electrical stimuli to individual brachial nerves recorded intracellularly in primary afferent axons in the dorsal horn in 0-Mg^{+2} Ringer solution.

68 brachial muscle spindle and cutaneous afferent axons were studied. In 88.5% of the cases, a single superthreshold stimulus to a peripheral nerve evoked PAD with an amplitude $\geq$ 50 μV. The rarity of PAD evoked by a single stimulus to a peripheral nerve in the cat reported by others might have been due to the presence of Mg^{+2} ions in the extracellular milieu. 20% of the 348 examples of PAD had a monosynaptic component that could not be accounted for by extracellular field potentials. Thus, some sensory afferents have direct synaptic connections with each other. Bicuculline (50 μM), a specific competitive antagonist of $GABA_A$ receptors, inhibited PAD by 54% showing directly that a major cause of PAD is activation of presynaptic $GABA_A$ receptors. This work was supported by NS86373 to E. F.

439.11

GABA INDUCED SPATIAL CHANGES OF CALCIUM INFLUX INTO NERVE TERMINALS D.W. Tank and K. Delaney* Research Dept. Molecular Biophysics AT&T Bell Laboratories Murray Hill, NJ 07974 and Dept. Physiol.-Anat., U.C. Berkeley, Berkeley, CA 94720.

Presynaptic inhibition of excitatory transmission at crayfish opener muscle synapses is mediated by gamma amino butyric acid (GABA). We have used fluorescence imaging to measure the effect of GABA on the spatial distribution of calcium influx in excitor nerve terminals during short stimulus trains (15s, 8-10 Hz). Excitor nerve terminals were filled with fura-2 by iontophoretic injection into the axon (15-20 nA, 30-60 min.). Calcium influx into terminals located on proximal and distal sections of a nerve branch was measured while recording the excitatory junction potential (EJP) in a muscle fiber. EJP's result from the summed activity of these spatially distributed terminals. Perfusion with 100-200 μM GABA reduced the EJP in a fiber near the imaged terminal by 85-100%. During this block terminals showed no increase in calcium but secondary and tertiary branches still showed near normal increases. Extracellular stimulating current required to initiate an action potential rose 20-30%. Lower GABA concentrations (10-50 μM) reduced EJP amplitudes by 40-60%. Terminals located distal to the main or secondary axons, or on the ends of fine branches, still showed no calcium increase during stimulation. However, terminals located near the main axon or on thick branches now showed normal increases in calcium. These data suggest that failure of the action potential to invade distal portions of the axonal arbor contributes significantly to the reduction of EJP amplitude by GABA. Our methods can be easily extended to examine the spatial distribution of calcium influx in the excitor axon during normal presynatic inhibition mediated by the inhibitor axon. Supported by NIH Grant NS 15114 and AT&T Bell Labs.

439.12

IDENTIFICATION OF THREONINE-286 AS THE AUTOPHOSPHORYLATION SITE IN THE α-SUBUNIT OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II (CaM K II) RESPONSIBLE FOR THE GENERATION OF CALCIUM-INDEPENDENT ACTIVITY.

G.Thiel*, A.J. Czernik*, F. Gorelick*, A.C. Nairn and P. Greengard, Lab. of Molecular and Cellular Neuroscience, The Rockefeller Univ., New York, NY 10021.

Autophosphorylation of CaM K II converts the enzyme to a calcium-independent form. The time course for conversion correlates with the autophosphorylation of a threonine residue contained within a thermolytic phosphopeptide common to both subunits (Lai et al.,PNAS,84:5710,1987). We have directly identified this site in the α-subunit of CaM K II by sequencing ^{32}P-labeled phosphopeptides derived from cleavage of the α-subunit. After autophosphorylation for 10 sec at 0°C to produce near-maximal calcium-independent activity, the α- and β-subunits were separated by SDS-PAGE. 600 μg of α-subunit protein was cleaved with CNBr for 72 hr and the fragments separated by C-18 HPLC using gradients ofCH_3CN in 0.1% TFA. A phosphopeptide (CB-1), containing 60% of the recovered radioactivity eluted at ~30% CH_3CN. CB-1 was further purified by C-4 and C-18 HPLC with shallow gradients in the same solvent system. Phosphoamino acid analysis of CB-1 revealed only ^{32}P-phosphothreonine. Using 20 pmol of CB-1, a sequence XRQETVDXLKKFNARRKL was obtained, which represents the N-terminal 18 residues of a 26-amino acid CNBr peptide predicted from the cDNA clone of the α-subunit and contains a consensus sequence for CaM K II phosphorylation which includes Thr-286. Two peptides (CB/C-1 and CB/C-2) derived from chymotryptic digestion of CB-1 were purified by C-18 HPLC. The sequences obtained for CB/C-1 and CB/C-2, XRQ(E/A)XVDXL and XXQETVD, respectively, confirmed that Thr-286 was the phosphorylated residue.

439.13

Dopamine receptor control of 3H-dopamine and 14C-acetylcholine release: Effects of phorbol ester and forskolin. Timothy W. Lovenberg*, Pamela A. Diliberto* and Luigi X. Cubeddu. Clin. Pharm., UNC, Chapel Hill, NC 27514

The Ca^{++}-dependent release of dopamine (DA) and acetyl choline (ACH) from the striatum is modulated by DA D2-receptors. However, the mechanism of this release inhibition is unknown. We have studied 3H-DA and 14C-ACh release from superfused rabbit striatal slices in the presence of phorbol-12,13-dibutyrate (PDBu) and forskolin (FSK) to determine if protein kinase C (PKC) and cAMP may play a role in the control of neurotransmitter release. PDBu enhanced the electrically stimulated, but not basal, release of 3H-DA in a concentration-dependent manner (EC50=10nM). 14C-ACh release was unaffected. In the presence of PDBu, DA D2 receptor agonists were less potent and less effective at inhibiting 3H-DA and 14C-ACh release. Also, the DA D2-receptor antagonist sulpiride was less effective at enhancing stimulated 3H-DA and 14C-ACh release. FSK also enhanced stimulated, but not basal release of 3H-DA (EC50=100nM). 14C-ACh release was unaffected. FSK did not alter the inhibition of 3H-DA and 14C-ACh release produced by D2-agonists.

These results suggest that, a) activation of adenylate cyclase and PKC may facilitate DA release and b) D2-receptor modulation of DA and ACh release may be regulated by PKC function.

439.14

INCOMPLETE AUTOPHOSPHORYLATION OF CALMODULIN-STIMULATED PROTEIN KINASE II (CMK II) IN INTACT SYNAPTOSOMES. A.Côté*, S.M. Harrison* and P.R. Dunkley*. (SPON: R. Lasher). The Neuroscience Group, Univ. of Newcastle, NSW, 2308 Australia.

When lysed synaptosomes are incubated with [γ-^{32}P]ATP the subunits of CMK II are major phosphoproteins, however when intact synaptosomes are incubated with $^{32}P_i$ very little autophosphorylation of these subunits occurs. Solubilized CMK II is susceptible to heat inactivation. However following a 45 min. incubation at 37°C and subsequent lysis and [γ-^{32}P]ATP labelling, minimal change of synaptosomal CMK II autophosphorylation and total CMK activity assayed using an exogenous substrate was observed. These results suggest that the intracellular environment of the synaptosomes is substantially different to the conditions used in in vitro assays. We have therefore examined the effects of different buffers and observed no significant change in the autophosphorylation of CMK II or exogenous peptide labelling when either Krebs or sucrose buffers were used, relative to low ionic strength Tris buffer. However, a small (20-30%) decrease in autophosphorylation was found in the presence of K^+ glutamate buffer. Thus, the lack of phosphorylation of intrasynaptosomal CMK II is not due to thermal inactivation and is only minimally contributed to by the intracellular ionic environment. (A. Côté is supported by the FRSQ of Quebec, Canada).

439.15

IN SITU PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN RAT STRIATAL SYNAPTOSOMES, BOVINE ADRENAL CHROMAFFIN CELLS, AND PC12 CELLS: DIFFERENCES IN PHOSPHOPEPTIDES. J.W. Haycock, M. Calalb* and D. Morgan*. Dept. Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70119.

Incubation of catecholaminergic tissues with $^{32}PO_4$ leads to the incorporation of ^{32}P into tyrosine hydroxylase (TH), the rate-limiting and initial step in catecholamine biosynthesis. Depolarization produces a calcium-dependent increase in the phosphorylation of TH at a number of sites; and, a number of protein kinase systems are potentially involved in mediating this effect.

In the present studies we compared the HPLC elution profiles of tryptic TH phosphopeptides from three tissues after prelabeling with $^{32}PO_4$ and treatment with a depolarizing stimulus (elevated $[K^+]_o$). Limit tryptic digestion of TH from each of the three tissues produced multiple TH phosphopeptides. Elevated K^+ increased ^{32}P incorporation into more than one peptide in each of the tissues. The retention of these phosphopeptides on a C_{18} column, developed with an acetonitrile gradient in 0.1% trifluoroacetic acid, was similar for the phosphopeptides from rat synaptosomes and PC12 cells. Only one of the TH phosphopeptides from bovine chromaffin cells eluted at a retention time which approximated that for any of the phosphopeptides from rat TH. Mixing of solubilized rat and bovine tissue, prior to immunoprecipitation, failed to reveal an identity of any of the bovine and rat TH phosphopeptides. The amino acid sequences of some of the tryptic peptides, as inferred from previously published cDNA sequences, differ at only one amino acid (gln vs leu; glu vs gln). The possibility that either genetic differences or tissue-specific, post-translational modifications may account for the observed differences in retention times is currently being investigated.

439.16

PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN RAT CORPUS STRIATUM *IN VIVO*: EFFECTS OF MEDIAL FOREBRAIN BUNDLE STIMULATION. Dean A. Haycock and John W. Haycock. Sterling Winthrop Res. Inst., Rensselaer, NY 12144 and Dept. Biochem. Molec. Biol., Louisiana State Univ. Med. Center, New Orleans, LA 70119

Tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, has been shown to be phosphorylated in a variety of preparations both *in vitro* and *in situ*. One essential criterion for determining the physiological significance of such findings is whether TH is phosphorylated *in vivo*. By infusing $^{32}PO_4$ (2 mCi, 5 ul, 60 min) and rapidly fixing the striata by liquid nitrogen injection (Neurosci. Abstr. (1986) 12:737), we have measured ^{32}P incorporation into TH *in vivo*. ^{32}P-TH, after immunoprecipitation and SDS-PAGE, showed no evidence of proteolysis. The TH bands were subjected to limit tryptic digestion, and the resulting phosphopeptides were separated by HPLC and analyzed with on-line radiochemical detection.

Tryptic digestion of TH labeled *in vivo* produced multiple phosphopeptides. The elution profile of these phosphopeptides was similar to that previously described for TH that was labeled *in situ* in striatal synaptosomes (Brain Res. Bull. (1987) 19:619). Electrical stimulation (biphasic square wave, 200 uA, 3 ms duration, 30 Hz, 20 min) of the medial forebrain bundle produced a change in the ratio of ^{32}P incorporation into two of the four major phosphopeptides. This change in the pattern of ^{32}P incorporation into TH phosphopeptides was different from that previously reported for TH in striatal synaptosomes depolarized with elevated $[K^+]_o$.

Thus, although similar TH phosphopeptides are observed both *in vivo* and *in situ*, the regulation of TH phosphorylation by electrical stimulation of the nigro-striatal pathway *in vivo* may differ from that produced *in situ*.

439.17

CHARACTERISTICS OF THE SPINAL CORD $5HT_{1B}$ AUTORECEPTOR DEFINED BY 5HT RELEASE INHIBITION AND [^{125}I]-IODOCYANOPINDOLOL BINDING. David J. Jones and Michelle Combs, Depts. Anesth. and Pharm., UTHSC, San Antonio, TX 78284.

Several groups have provided evidence that the 5HT autoreceptor is of the $5HT_{1B}$ type. Recent studies have used [^{125}I] - iodcyanopindolol ([^{125}I]-CYP) to label the $5HT_{1B}$ receptor in various areas of the CNS. The purpose of the present work was to pharmacologically characterize both the autoreceptor linked to 5HT release regulation and that defined by [^{125}I] - CYP binding in the spinal cord.

Synaptosomes labeled with [^{3}H]-5HT were superfused with control buffer or buffer containing 15 mM K^+ to stimulate release. [^{125}I]-CYP binding was carried out in the presence of 30 uM isoproterenol to occlude beta receptors and 100 nM 8-OH DPAT to occlude $5HT_{1A}$ receptors. Specific binding was defined in the presence of 10 uM 5HT and was 60-80% of total binding.

The rank order of potency of 5HT agonists for the inhibition of K^+ stimulated [^{3}H]-5HT release was CGS 120668 > 5HT > RU 24969 > quipazine = 2mPP > mCPP > TFMPP. The rank order of potency for displacement of specific [^{125}I]-CYP was CGS 120668 = 5HT > RU 24969 > 2 mPP > TFMPP > mCPP = quipazine. 8-OHDPAT (< 10 uM) was inactive in both systems. Based on this similar ranking, [^{125}I]-CYP binding may predict for the functional component of the $5HT_{1B}$ site in spinal cord. Supported by NIH grant NINCDS 14546.

440.1

DIFFERENTIAL EFFECTS OF d-MEDETOMIDINE ON ^{3}H-DOPAMINE RELEASE FROM RAT STRIATAL AND ACCUMBENS SYNAPTOSOMES. J.G. Csernansky, E.C.Petrie*, D. Blanchard*, D. Ott*, and R. Lammintausta*. Schizophrenia Biologic Research Center, Palo Alto VA Medical Center and Dept. of Psychiatry & Behav. Sci., Stanford Univ., Stanford, CA 94305.

DA neurons in the neostriatum (NS) possess inhibitory presynaptic receptors (heteroreceptors) stimulated by other neurotransmitters. Clonidine has been shown to inhibit potassium (K^+) stimulated ^{3}H-DA release from rat nucleus accumbens (NA) slices, suggesting the presence of alpha-2 heteroreceptors in that region as well. We assessed the effects of the alpha-2 agonist d-medetomidine on K^+ stimulated ^{3}H-DA release from rat NA and NS synaptosomes in a continuous superfusion system. In NA, control release of the total was 18.6±1.1% (mean ±SEM) of ^{3}H-DA initially present in the synaptosomes. Unlabelled DA (1.8uM) reduced release to 13.1 ± 1.4% (t=3.1, p=0.01 vs. control) consistent with an action on autoreceptors. Similarly, d-Medetomidine (1.0uM) reduced ^{3}H-DA release to 13.6±1.0% (t=3.1, p=0.013 vs. control), an effect comparable in magnitude to that produced by DA. In NS, control release of ^{3}H-DA was 25.7±0.6% and unlabelled DA (1.8uM) reduced release to 16.8±0.5% (t=11.02, p<.001 vs. control). d-Medetomidine (1.0uM) had little effect on ^{3}H-DA release in the NS, with a mean ± SEM of 25.1±0.8%, not significantly different from the control value. Our data suggest that alpha-2 heteroreceptors are present on only a subset of central DA nerve terminals and imply that it may be possible to selectively modify central DA activity with alpha-2 agonists and antagonists.

440.2

FACILITATORY AND INHIBITORY TRANSMITTERS MODULATE SPONTANEOUS RELEASE AT CULTURED APLYSIA SENSORIMOTOR SYNAPSES. N. Dale* and E.R. Kandel (SPON: C.A. Kaufmann). HHMI, Columbia CPS, New York, NY 10032

Two independent presynaptic mechanisms, one involving modulation of ionic currents and the other an increase in the availability of transmitter for release, contribute to heterosynaptic facilitation at the sensorimotor synapses of Aplysia. To study the second of these mechanisms, we observed the spontaneous miniature epsps at synapses formed between single sensory and motor cells in vitro. 1-10 uM 5-HT reversibly increased the frequency of spontaneous release 4-5 times while the inhibitory peptide FMRFa (100 nM) reversibly reduced the frequency of spontaneous release to near control values when applied simultaneously with the 5-HT. A major component of the modulation of spontaneous release does not depend on either a Ca influx or changes in the concentration of internal Ca. In salines lacking Ca or containing 1 mM Cd to block Ca currents, 5-HT still enhanced the rate of release by 3-4 times, and FMRFa when applied simultaneously reduced the rate to near control values. When BAPTA was injected into the sensory neuron to buffer the internal Ca concentration, 5-HT and FMRFa could still modulate spontaneous release. The rate of spontaneous release, and consequently the transmitter available for evoked release during presynaptic inhibition and presynaptic facilitation, can therefore be modulated by 5-HT and FMRFa directly by a mechanism which is independent of changes in internal Ca.

440.3

IMMUNOAFFINITY ISOLATION OF HIPPOCAMPAL NERVE TERMINALS BEARING [^{3}H]NICOTINE RECEPTORS. J.Irons*, G.G.Lunt, S. Wonnacott, P.Whiting† & J.M.Lindstrom†. Dept.Biochem., Univ.Bath, BATH, BA2 7AY U.K. & †Salk Inst., P.O.Box 85800, San Diego, CA92138 U.S.A.

Nicotine stimulates the release of [^{3}H]GABA and [^{3}H]ACh from hippocampal synaptosomes.The presynaptic nicotinic acetylcholine receptor (nAChR) mediating this action has a pharmacological specificity similar to that of the high affinity binding sites for [^{3}H]nicotine in brain membranes.Using monoclonal antibodies (mAbs) 270 and 290 which are specific for these nAChR, we have immunoaffinity isolated synaptosomes bearing surface [^{3}H]nicotine receptors.Synaptosomes, highly purified from rat hippocampus by isotonic Percoll density gradient centrifugation, were enriched in [^{3}H]nicotine binding sites and high affinity transport of [^{3}H]GABA and [^{3}H] choline.Synpatosomes were incubated with mAbs 270 and 290 coupled to Dynabeads M-450 (Dynal) and bound synaptosomes were separated magnetically.Up to 25% of the total nerve terminal population could be immunoisolated, as judged by measurement of lactate dehydrogenase.A differential purification of cholinergic and GABAergic terminals, assessed by CAT and GAD activities, respectively, was observed. These results suggest that nAChR are present on 25% of rat hippocampal nerve terminals, including cholinergic and GABAergic terminals, consistent with the presynaptic modulation of transmitter release by these nAChR. (Supported by R.J.Reynolds Co.)

440.4

TRANSDUCTION OF THE MODULATORY EFFECT OF NOREPINEPHRINE ON TRANSMITTER RELEASE AT MOTOR NERVE TERMINALS. H. Chen,* W.F. Dryden and Y.N. Singh* Dept. of Pharmacol., Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

There is general agreement that the enhancement of neurotransmitter release from motor nerve terminals by norepinephrine (NE) is mediated by α_1-adrenoceptors (Chen and Dryden, Neurosci. Abst. 13:68, 1987). However Miyamoto and Breckenridge (J. Gen. Physiol. 63:609, 1974) suggested that the response involved a cyclic-AMP link and this conflicted with existing concepts of transduction at the α_1-adrenoceptor. The present study was undertaken to identify the transduction mechanism involved at this site. MEPP frequency was measured at 20°C in mouse hemidiaphragms exposed to 15mM$[K^+]_0$. The enhanced release of quanta caused by 1μM NE was reduced by nonspecific kinase inhibitors (50μM clomiphene, 1.2 μM polymyxin B, 20μM auranofin) but was not affected by 50 μM H_7, an inhibitor of cyclic nucleotide dependent kinase and protein kinase C. It was reduced by 10μM W_7, an inhibitor of calcium-calmodulin dependent (CaM) kinases. The effect of NE was increased by 10mM Li, which prevents further metabolism of inositol triphosphate (IP_3), but was unaffected by pertussis toxin (2.5 μg/ml for 4 hours at 37°C). These results are consistent with transduction by IP_3 to cause intraterminal Ca^{2+} release and activation of a CaM kinase.
Supported by Univ. of Alberta Central Research Fund.

440.5

NORADRENALINE (NA) MEDIATES A COMPONENT OF VIP RELEASE EVOKED BY 4-AMINOPYRIDINE (4-AP) IN MOUSE NEOCORTEX. J.L. Martin and P.J. Magistretti. Département de Pharmacologie, CMU, 1211 Geneva 4, Switzerland.

In mouse cerebral cortical slices, 4-AP stimulates in a concentration-dependent manner basal VIP release (VR). VR evoked by 4-AP 1 mM is completely blocked by Co^{++} 2.5 mM and partially inhibited (74 %) by TTX 2 μM. Mepacrine, an inhibitor of phospholipase A_2, inhibits 4-AP-evoked VR (4-APVR) with an IC_{50} of 15 μM. Indomethacin, a cyclooxygenase inhibitor, and caffeic acid, an inhibitor of 5-lipoxygenase, inhibit by 35 % (at 100 μM) and 67 % (at 1 mM) respectively, 4-APVR. These inhibitors of arachidonic acid metabolites' formation act exclusively on the TTX-sensitive component of 4-APVR. NA potentiates in a concentration-dependent manner (between 1 and 100 μM) 4-APVR, while not affecting basal VR. Furthermore, 4-APVR is inhibited by the α-2-adrenergic antagonist yohimbine, but not by prazosin, an α-1-adrenergic antagonist. These results indicate that part of 4-APVR may be mediated through the release of NA. VR is also stimulated in a concentration-dependent and TTX-insensitive manner by K^+ (between 15 and 50 mM). K^+-evoked VR is completely inhibited by Co^{++} 2.5 mM and partially by Mn^{++} 1 mM (75 %) and Ni^{++} 0.1 mM (34 %) but not by Cd^{++} 20 μM. Diltiazem 20 μM, nifedipin 10 μM and ω-conotoxin 1 μM do not inhibit VR. This pharmacological profile suggests the involvement of voltage-sensitive Ca^{++}-channels of the T subtype in K^+-VR. Supported by FNRS Grant N° 3.357-0.86.

440.6

LITHIUM ENHANCES PILOCARPINE-PRODUCED EPILEPTIFORM ACTIVITY BY PRESYNAPTIC FACILITATION. M.S. Evans*, C.F. Zorumski, and D.B. Clifford. Depts. of Neurology and Psychiatry, Washington University Sch. of Med., St. Louis, MO 63110.

Pilocarpine (PILO)-injected rats develop limbic seizures; its potency is increased about 20-fold by lithium. This interaction was investigated in hippocampal slices. Slices were taken from 150-350 g rats, incubated in an oxygenated chamber and transferred as needed to a submerged-type chamber where they were perfused with oxygenated medium (NaCl 116.4, KCl 2.0, $MgSO_4$ 1.3, $CaCl_2$ 1.5, NaH_2PO_4 1.0, $NaHCO_3$ 26.2, glucose 11). Drugs were applied via the perfusate. Intracellular and field potentials were recorded in area CA1 with stimulation of Schaffer collaterals. PILO had effects like other muscarinic agonists: it increased input resistance, blocked a slow AHP, blocked accomodation to depolarizing current pulses, and depolarized the cell slightly at 100-1000 nm. Lithium 1-5 meq/l either did not change or slightly reduced these effects of PILO. Lithium increased the amplitude of dendritic field potentials (DFP) 100% at 5 meq/l, 18% at 1 meq/l, and enhanced intracellular EPSPs as well, but the response to iontophoretic glutamate pulses was unchanged or decreased, suggesting its effect is presynaptic. PILO reduced EPSPs and DFPs by presynaptic inhibition. In the presence of 500-1000 nm PILO, lithium 1 meq/l increased DFPs by 50-100%. Repetitive epileptiform spiking was routinely produced with the combination of drugs, but infrequently by PILO alone. These results suggest that lithium-PILO seizures result from an interaction at a presynaptic level, with small concentrations of lithium potently antagonizing PILO's presynaptic inhibitory effect.

440.7

INTERACTIONS OF MDMA STEREOISOMERS ON HIGH AFFINITY UPTAKE IN RAT HIPPOCAMPAL SYNAPTOSOMES. Poblete, J.C., Avdelis, B.J., Whitaker-Azmitia, P.M., Azmitia, E.C. (SPON: Feinberg, I.) Dept. Biology NYU, New York, NY 10003 and Dept. Psychiatry, SUNY, Stony Brook, Stony Brook, NY 11794.

We investigated the interactions of the (R) and (S) enantiomers of MDMA on rat hippocampal synaptosomes with respect to uptake of ^{3}H-5HT (50 nM) as previously described (Azmitia and Whitaker-Azmitia, 1987). In the first series of studies, (R) and (S) MDMA (10^{-11}M - 10^{-4}M) were added with and without Ca^{++} (incubation 37°, time = 30 min.). In our second series of tests, we performed an experiment to determine whether the enantiomers exerted their effects through the same transporter site.

The (R) isomer exhibited a more profound inhibition of 5HT uptake than the (S) isomer at identical concentrations [43% for (S) vs. 68% for (R) at 10^{-7}M]. Both isomers acted in a dose dependent manner with significant reduction at nanomolar concentration. In low Ca^{++} media, the uptake of 5HT was 50% lower after 30 minutes, and both isomers produced almost complete inhibition at 10^{-9}M. Our results from the second series of studies showed that the enantiomers of MDMA compete for the same transport site since the (S) isomer blocked the greater inhibition produced by the (R) isomer, and no evidence of cooperativity was found. These data suggest that one high affinity binding site exists for both enantiomers of MDMA, and the (R) isomer has a more potent effect of 5HT uptake in rat hippocampal synaptosomes. Supported by NIDA contract 271-87-8144.

440.8

PREVENTION OF MDMA TOXICITY TO SEROTONERGIC NEURONS IN TISSUE CULTURE BY L-TYPE CA^{++} CHANNEL ANTAGONIST. Azmitia, E.C., Murphy, R.B., Whitaker-Azmitia, P.M. Dept. Biology and Chemistry, NYU, New York, NY 10003 and Dept. Psychiatry, SUNY, Stony Brook, NY 11794.

We report that S(+)- MDMA is stimulatory at low concentrations (around 10^{-9}M) and inhibitory at high concentrations (above 10^{-6}M) on the expression of [^{3}H]-5-HT uptake on cultured serotonergic neurons. The R(-)- MDMA was inhibitory within both concentration ranges. Multiple applications of MDMA enhanced the inhibitory effects of the R enantiomer. This differential effect of the enantiomers at low concentration was confirmed with morphometric analysis of 5-HT neurons grown in normal or no Ca^{++} media. The S-MDMA enantiomer produced increases in the number of surviving 5-HT neurons, their somal area and process length. In contrast, the R-MDMA at low concentrations produced a decrease in somal area and process length of 5-HT neurons.

The specific L-channel Ca^{++} antagonist, nimodipine (2×10^{-5}M), blocked the inhibitory action of both the R and S enantiomers at high concentrations of MDMA (10^{-3} - 10^{-2}M), but did not interfere with the differential effects seen at low concentrations on the expression of reuptake by the cultured serotonergic neurons. Our results indicate that MDMA toxicity (as operationally defined) is due to an excess influx of Ca^{++} through an L-type Ca^{++} channel which appears to be independent of the biological stimulatory effects of S-MDMA on serotonergic neurons in culture. Thus, if the L-type channel mediated Ca^{++} influx is inhibited, S-MDMA appears paradoxically to function as a neuronotrophic factor since it can increase cell survival, induce process outgrowth and enhance the high affinity uptake of [^{3}H]-5-HT. The present mechanism may be of general importance in the mediation of actions of other neurotoxins. Supported by NIDA contract 271-87-8144.

440.9

MODULATION OF GLUTAMATE AND DYNORPHIN RELEASE FROM HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMES. R.L. Gannon, L.T. Baty* and D.M. Terrian. Clinical Sciences Div., USAF Sch. Aerospace Med., Brooks AFB,TX 78235-5301.

The long-term potentiation (LTP) of the hippocampal mossy fiber (MF)-CA3 synaptic response is not sensitive to 2-amino-5-phosphonovalerate as is the induction of LTP at other hippocampal synapses, but this synaptic input can be selectively and potently blocked by 2-amino-4-phosphonobutyrate (APB). This effect is not accompanied by a change in postsynaptic excitability and, therefore, may be mediated by the presynaptic inhibition of transmitter release from the MF terminals. To examine this possibility we have studied the effects of APB on the K^+-evoked release of L-glutamate (Glu) and dynorphin from a subcellular fraction enriched in hippocampal MF synaptosomes. Endogenous Glu and dynorphin A(1-8) release were determined fluorometrically and by radioimmunoassay, respectively. A two minute application of 45 mM KCl to superfused guinea pig synaptosomes evoked a release of 55.7 ± 3.5 pmol Glu/min/mg protein that was partially Ca^{2+}-independent. L(+)APB, (300 µM) reduced the KCl-evoked release of dynorphin A(1-8) from 1.12 ± 0.07 to 0.53 ± 0.1 pg/min/mg protein, but had no effect on Glu release. However, after exchanging Glu out of the cytosolic pool with 50 µM D-aspartate, 300 µM L(+)APB reduced the remaining Ca^{2+}-dependent Glu release from 47.4 ± 3.1 to 29.8 ± 3.7 pmol/min/mg protein. Under the same conditions L(+)APB had no significant effect on Glu or dynorphin A(1-8) release from rat MF synaptosomes.

440.10

ADENOSINE INHIBITION OF GLUTAMATE AND DYNORPHIN RELEASE FROM HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMES. P.G. Hernandez*, R.L. Gannon, M.A. Rea and D.M. Terrian. USAF School of Aerospace Medicine, Brooks AFB, TX 78235-5301.

Electrophysiological studies indicate that the anticonvulsant effect of adenosine and its analogues on hippocampal CA3 pyramidal neurons is primarily due to a presynaptic inhibition of excitatory neurotransmitter release. The hippocampal mossy fiber (MF) system provides a major excitatory synaptic input to the CA3 subfield and may represent a site of adenosine action. We have recently reported that the adenosine agonist 2-chloroadenosine (ClAdo) preferentially inhibits the Ca^{2+}-dependent component of glutamic acid released by a synaptosomal preparation enriched in hippocampal MF nerve endings. In the present experiments, we have investigated the mechanism by which adenosine modulates the MF synapse. The results demonstrate that ClAdo (0.1 µM) inhibits the 45 mM K^+-evoked release of dynorphin A(1-8), as well as glutamic acid, from superfused MF synaptosomes. Exogenous ATP (0.1 - 1.0 mM) does not mimic this effect and the order of agonist potency is ClAdo ≈ cyclohexyladenosine > 5'-N-ethylcarboxamidoadenosine. The IC_{50} for the effects of ClAdo on glutamic acid release is near 0.1 µM and this inhibition can be prevented by increasing the concentration of calcium from 0.5 to 7.0 mM. These findings suggest that adenosine presynaptically modulates the hippocampal MF synapse by interfering with calcium influx or its availability within the nerve terminal.

SENSORY SYSTEMS: AUDITORY SYSTEMS VII

441.1

AUDITORY LOCALIZATION IN THE SAW-WHET OWL. M.L. Csizy*, B.J. Frost. Queen's University, Kingston, Ontario K7L 3N6.

The Saw-whet owl (Aegolius acadicus) is a nocturnal raptor which acoustically locates its prey with great accuracy. The auditory localization abilities of this species has been studied using head orientation responses to sounds presented in a free field.

Employing the search coil technique (Robinson, 1963; Knudsen et al, 1979) permitted the tracking of lateral and vertical head motions in response to playback sounds of mice moving through leaves. Saw-whets showed the greatest accuracy for sounds emanating from a region directly in front, spanning 40 degrees in each dimension. The average error was less than 1 degree in azimuth and just above this in elevation. Acuity was reduced at the extreme limits of the sound field, the mean error for speakers positioned at 30 and -30 degrees along each axis being 6 degrees. A decline in accuracy for sounds originating from more than 10 degrees above the owl's interaural axis may be a function of the biological irrelevance of the particular stimulus at these elevations.

Testing to determine the independent contribution of interaural time and intensity differences localization is in progress. This research was supported by an NSERC grant A0353 to B.J.F.

441.2

THE REPRESENTATION OF SOUND FREQUENCY AND SPACE IN THE MIDBRAIN OF THE SAW-WHET OWL (*Aegolius Acadicus*). L.Z. Wise, B.J. Frost and S.W. Shaver. Dept. of Psychology, Queen's University, Kingston, Ont. K7L 3N6 Canada.

We studied frequency and spatial tuning of single cells in the midbrain auditory nucleus (MLD) of the ketamine-anesthetised saw-whet owl. This bird has a pronounced asymmetry in the bone structure of the ear openings, and has extremely acute sound localization ability (Csizy and Frost, *Neurosci. Abstr.*, 1988). Cells in central MLD were sharply tuned to frequencies from 200 Hz, represented dorsally, through 7 kHz, represented ventrally, and exhibited little or no sensitivity to sound source location. Cells in lateral MLD (MLD1) were broadly tuned to frequency, responded vigorously to noise stimuli, and were spatially selective. From rostral to caudal MLD1, cells preferred increasingly contralateral locations. Azimuthal spatial tuning was sharper than elevational tuning, and matched sensitivity to interaural time delay recorded in the same cells using removable headphones. Auditory responses were also recorded in the optic tectum overlying MLD. Cells responded preferentially to wide-band noise stimuli, and showed spatial selectivity in both azimuth and elevation. Supported by NSERC Grant A0353 to B.J.F.

441.3

THE RELATION BETWEEN VISION AND SOUND LOCALIZATION ACUITY IN MAMMALS. R. S. Heffner and H. E. Heffner. Dept. of Psychology, Univ. of Toledo, Toledo, OH 43606.

It has long been noted that one function of hearing is to direct the eyes to the source of a sound. Recent analysis suggests that this function may be the major source of selective pressure on sound localization acuity in mammals.

If the ears are to direct the eyes to the source of a sound, then the question arises as to how accurate the ears must be. Since humans direct their area of best vision (i.e., their fovea) to the source of a sound, we reasoned that the acuity of sound localization needed to direct the eyes might be related to the size of an animal's field of best vision.

Using retinal ganglion cell counts we defined the size of the "area of best vision" as the horizontal extent (in degrees) of the region encompassing cell densities greater than or equal to 75% of maximum density. We then correlated area of best vision with sound localization acuity in the 8 species for which both measures were available (opossum, human, gerbil, grasshopper mouse, weasel, cat, pig, cattle). The resulting correlation was 0.96 ($p<.01$).

This result suggests that the need to visually locate a sound source may be the primary source of selective pressure on sound localization acuity and may account for the wide variation in sound localization acuity in mammals.
(Supported by NIH grant NS 17850)

441.4

LOCALIZATION OF UNDERWATER SOUND IN THE CLAWED FROG, XENOPUS LAEVIS. B.SCHANZ*, A.ELEPFANDT (SPON: European Brain and Behaviour Society). Fak. Biol., Univ. Konstanz, P.O.Box 5560, D-7750 Konstanz, Fed. Rep. Germany.

Localization of underwater sound was investigated in the aquatic clawed frog, Xenopus laevis. Previously, it had been argued that binaural mechanisms - such as used by terrestrial frogs - are insufficient for locating low-frequency underwater sound (van Bergeijk, W.A., in: Tavolga, W. (ed), Marine bio-acoustics. Pergamon, New York, 281,1964).

Adult females injected with gonadotropin were tested individually in a basin 4 m in diameter filled with water to a depth of 30 cm. Male mating calls were presented from various directions through underwater loudspeakers and the angles of the turning responses were measured as a function of sound direction.

The animals located sound from anterior and lateral directions with an accuracy of $\pm 19°$, which is only slightly poorer than in terrestrial frogs. Localization of posterior sound was considerably worse.

Animals with unilateral destruction of tectum and torus responded to contralateral sound by turning ipsilaterally, which indicates a lateralization of sound localization in the midbrain.

441.5

TWO-DIMENSIONAL SOUND LOCALIZATION BY HUMAN LISTENERS. J.C. Middlebrooks and J.C. Makous*. Depts. of Neuroscience and Surgery (ENT), University of Florida, Gainesville, FL 32610.

We measured the ability of human listeners to localize white noise bursts (150 msec duration) presented at horizontal and vertical locations distributed throughout nearly 360° of auditory space. Each subject reported the location of the sound source by orienting toward it while his head position was monitored with an electromagnetic device. Each sound source location was tested 5 or more times.

Subjects effectively discriminated among source locations separated by horizontal and vertical steps of 10° throughout most of the frontal half of space. Localization was most precise for midline sources (standard deviations around 2°), but the precision of localization typically fell by no more than a factor of 3 for locations in the frontal 180° within 35° of the horizontal plane. Front/back errors were infrequent. Localization precision was substantially decreased for sounds presented behind the subject; in part, this may reflect the difficulty in turning to face a sound presented from behind.
(UF award DSR-D-54 and NIH grant R29 NS25022)

441.6

GABA-MEDIATED INHIBITION CONTRIBUTES TO NEURONAL SELECTIVITY FOR INTERAURAL TIME DIFFERENCE IN THE OWL'S INFERIOR COLLICULUS. I. Fujita* and M. Konishi (SPON: D. Felleman). Biol. Div., 216-76, Calif. Inst. of Technol., Pasadena, CA 91125.

The barn owl uses interaural time differences (ITD) for localizing the azimuthal position of sounds. Neuronal selectivity for ITD first appears in the nucleus laminaris and improves in the central nucleus "core" (ICc) and the external nucleus (ICx) of the inferior colliculus. Tonal stimuli cause both ICc and ICx neurons to respond maximally not only to one particular ITD (the characteristic delay), dt, but also to dt + nT, where T is the tonal period and n an integer. This phenomenon, phase ambiguity, does not occur when ICx neurons are stimulated with noise.

Iontophoretically applied bicuculline methiodide (BMI, a selective $GABA_A$ antagonist) decreased the ITD selectivity of ICc neurons. The effects were identical for tone- and noise-evoked responses. In ICx, BMI decreased ITD selectivity to tones only in neurons tuned to frequencies below 5 kHz. BMI led to loss of ability of ICx neurons to signal uniquely their dt. The results suggest that under physiological conditions GABAergic inhibition sharpens ITD selectivity in ICc neurons and ICx neurons tuned to below 5 kHz, and eliminates phase ambiguity in ICx by interaction between the converging frequency bands. (Supported by NIH and Uehara Memorial Foundation.)

441.7

IN VITRO PHYSIOLOGICAL STUDIES OF A "PLACE MAP" IN N. LAMINARIS OF THE CHICK. E. M. Overholt*, R. L. Hyson*, and E. W Rubel (SPON: D. Sutton). Depts. of Otolaryngology and Physiology/Biophysics, Univ. of Washington School of Medicine. Seattle, WA 98195.

Third order auditory neurons in the avian n. laminaris (NL) are the first to receive binaural input. In the chick, NL consists of a monolayer of neurons with polarized dendritic arbors oriented dorsally and ventrally. Afferents from the ipsilateral 2nd order neurons in n. magnocellularis (NM) innervate the dorsal dendrites of NL while the ventral dendrites receive afferents from the contralateral NM via the crossed dorsal cochlear bundle. The arborization pattern of axons arising from contralateral and ipsilateral NM are arranged in such a way that they could provide a differential delay network creating a "place map" within NL which corresponds to the time delay of sounds arriving at the two ears (Young, S. R. and E. W Rubel, J. Neurosci., 3:1373, 1983).

We tested this possibility by analyzing evoked field potentials using an *in vitro* slice preparation containing both NL and NM. Field potentials recorded while stimulating the contralateral NM or crossed dorsal cochlear bundle demonstrated a nearly linear increase in the latency of postsynaptic potentials from medial to lateral positions in NL. Conversely, when stimulating the ipsilateral auditory nerve stump or ipsilateral NM, the latency of the post-synaptic response showed no consistent variation. This differential delay circuit provides a mechanism for converting time delay of stimuli reaching the two ears into a "place map" of simultaneous postsynaptic activation of NL cells. (Support provided by PHS grant NS 07246 and NIH grant NS 24522.)

441.8

THE EFFECTS OF SUPERIOR OLIVARY COMPLEX LESIONS ON BINAURAL INTERACTION IN THE AUDITORY CORTEX OF THE ALBINO RAT. S. L. Sally* and J. B. Kelly. Lab. of Sensory Neuroscience, Dept. of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Cells in the superior olivary complex of the adult albino rat were selectively destroyed by micro-injections of kainic acid. Following a 2-3 week recovery period, the binaural response properties of cells in auditory cortex were examined using microelectrode mapping techniques. Rats were anesthetized with Equithesin (3.0 ml/kg i.p.), the cortical surface was exposed, and recordings were made with tungsten microelectrodes. Pure tone pulses were delivered to each ear independently through sealed loudspeakers fitted to specula inserted into the external meatus. Sound pressure levels were determined by probe tube measurements within a few mm of the tympanic membrane. Following complete unilateral ablation of the superior olivary complex, the three major binaural response types (summation, suppression and mixed) were represented at all frequencies within the rat's hearing range. The majority of neurons exhibited binaural interaction at interaural intensity differences (IIDs) which were within the normal range. Maps of auditory cortex ipsilateral or contralateral to the lesion showed the same pattern of results. Complete bilateral ablation of the superior olivary complex, however, resulted in a preponderance of cells sensitive only to monaural contralateral stimulation. There was a substantial increase in the IID thresholds of cells which did exhibit binaural interactions.
(This research was supported by NSERC operating grant 7654 to J.B.K.)

441.9

BINAURAL INTERACTION IN THE BRAINSTEM AUDITORY EVOKED RESPONSE OF THE FERRET (MUSTELA PUTORIUS). G.L. Kavanagh*, J.B. Kelly and T.W. Picton (SPON: E. Peterson). Lab. of Sensory Neuroscience, Carleton University, Ottawa, Canada, K1S 5B6 and Human Neurosciences Research Unit, University of Ottawa, Ottawa, Canada.

The effect of binaural interaction on the BAER was examined in nine adult male ferrets. Animals were anesthetized with pentobarbital sodium (35 mg/kg i.p.), placed within a sound-proof chamber, and the head was immobilized using a nontraumatic nose clamp. Clicks were produced by a 0.1 msec square wave, amplified and transduced by earphones over a broad range of intensities (34 to 104 dB peak SPL). Platinum needle electrodes were placed over the vertex and left and right mastoid. Responses were amplified 10,000 times, bandpass filtered between 0.3 Hz and 10 kHz and averaged on line at a rate of 50 kHz. The analysis epoch for each channel was 10.24 msec and all responses were based on 1,000 sweeps. The binaural interaction component of the BAER was derived using the procedure of Dobie and Berlin (1979). At each intensity the responses to left and right monaural stimulation were summed to obtain a predicted binaural response. The predicted response was then subtracted from the binaural response generated by stimulating both ears simultaneously. This yielded a difference trace containing the binaural interaction component. Binaural interaction in the ferret BAER is characterized by a prominent negative wave with a latency similar to the fourth vertex-positive potential (P4). The wave showed an increase in latency and reduction in amplitude as sound intensity was reduced over a 70 dB range.

441.10

INFLUENCE OF STIMULUS SPECTRUM ON AZIMUTH SELECTIVITY OF SINGLE UNITS IN CAT AUDITORY THALAMUS. W.A. Irons*, E.R. Samson*, T.J. Imig, Dept. of Physiology, Univ. Kansas Med. Ctr., Kansas City, KS 66103.

Single units in the ventral nucleus and Po of barbituate anesthetized cats were studied using free field stimulation. A series of tone bursts, broad band (BBN), or band pass (BPN) white noise bursts which systematically varied in level was delivered from each of several azimuths. Isorate contours were computed and plotted in SPL or spectrum level vs. azimuth coordinates. On the basis of these contour plots, three classes of response patterns were distinguished. 1) Nondirectional. These units were broadly tuned to azimuth for BBN, BPN, and tones. 2) Azimuth-selective, bandwidth-insensitive. These units were azimuth selective, and the degree of azimuthal selectivity and discharge rate was similar for BBN, BPN, and tones. Two-tone stimulation resulted in little evidence of sideband interaction. 3) Azimuth-selective, bandwidth-sensitive. These units displayed highly directional tuning to BBN with decreasing selectivity as the spectral bandwidth narrowed. Units often exhibited broad azimuthal tuning to tonal stimuli, however some units were more directionally tuned to some frequencies than others. Unit discharge rate was lowest to broad band stimulation, and highest to narrow band stimulation centered within the frequency response area suggesting the existence of inhibitory sidebands. Two-tone stimulation showed direct evidence of inhibitory and facilitory sidebands, the former being much more common. Some units displayed inhibitory sidebands on both sides of the excitatory response area, while others exhibited only one inhibitory sideband. The degree of sideband inhibition appears to be a function of both stimulus level and direction. Sideband inhibition appears to play a role not only in shaping a unit's frequency selectivity, but also its directional selectivity. (Supported by NINCDS Grant 17220 and BRSG S07RR05373)

441.11

PROCESSING OF INTERAURAL LEVEL DIFFERENCES IN THE INFERIOR COLLICULUS OF THE BARN OWL. R. Adolphs* (SPON: M. Konishi). Biology Div., 216-76, California Institute of Technology, Pasadena, CA 91125.

Barn owls use interaural differences in sound pressure level (ILD) to compute location of the source in elevation. I investigated the origin of neuronal selectivity for ILD in the medial shell of the central nucleus of the inferior colliculus (MS), a presumptive input stage to spatial elevation coding in the external nucleus (ICx).

Anatomical connectivity was demonstrated by retrograde labelling with horseradish peroxidase. Neurons of MS receive direct massive projections from the contralateral nucleus angularis, nucleus ventralis lemnisci lateralis pars posterior (VLVp), lateral region of the superior olive, and sparse projections from contralateral MS.

All above nuclei show stimulus-response curves sensitive to ILD and generally insensitive to interaural time differences (ITD), culminating in typically very sharp "EI" response curves in MS neurons: they are excited by ILD's favoring the contralateral ear, and inhibited by ILD's favoring the ipsilateral ear. Preliminary data from lidocaine injections into the projecting nuclei suggest that both VLVp and MS make inhibitory contralateral connections to MS. This is consistent with predictions made on the basis of connectivity and ILD-response character.

441.12

NEURONS IN THE INFERIOR COLLICULUS RECEIVE CONVERGENT INPUT FROM MULTIPLE BINAURAL SOURCES. R. Batra, S. Kuwada and T.R. Stanford*. Dept. of Anatomy, Univ. of Conn. Health Cntr., Farmington, CT 06032.

Several nuclei in the auditory pathways that receive input from both ears project to the inferior colliculus (IC). These include the nuclei of the superior olive, lateral lemniscus, and the auditory cortex. Several of these nuclei project to the same region of the IC, but do these projections converge on the same neurons? Here we report that single neurons in the IC which are sensitive to interaural temporal disparities (ITD's) appear to receive inputs from several binaural sources.

We recorded from neurons in the IC of the unanesthetized rabbit that were sensitive to ITD's of the waveform or the envelope. Calibrated pure and sinusoidally amplitude modulated tones were delivered dichotically. Responses were assessed at several tonal or modulation frequencies.

Neurons sensitive to ITD's are thought to arise from the convergence of phase-locked signals from the two ears along pathways which have fixed delays. Such a mechanism predicts a linear relationship between the interaural phase difference to which a neuron is most sensitive (the mean phase) and the stimulating frequency, with the slope representing the difference in delays from the two ears. We have observed significant deviations from this expected linearity in the IC. These deviations can take many forms. They can appear as oscillations of the mean phase around the regression line, or as a separation of the mean phase *vs.* frequency plot into two segments with different slopes. The two slopes indicate that the neuron receives convergent input from two sources, each of which processes a different ITD over a different range of frequency. Responses can also exhibit nulls at intermediate frequencies. All of these effects suggest convergence from two or more sources, each of which in turn receives phase-locked inputs from the two ears.

This research was supported by NINCDS grant NS-18027.

441.13

BINAURAL INTERACTION OF MIDDLE AND LONG-LATENCY AUDITORY EVOKED POTENTIALS D. L. Woods and C. C. Clayworth*, Clinical Electrophysiology Laboratory, Dept. of Neurology, U.C. Davis, VA Medical Center, Martinez, CA, 94553

We studied the binaural interaction of middle (latencies 10-70 msec) and long-latency (latencies 70-300 msec) auditory evoked potentials (AEPs). Stimuli were presented monaurally to the left and right ears and binaurally in different blocks while EEG recordings were obtained from 16 scalp sites.

The shortest latency evidence of binaural interaction was a partial occlusion of the Pa component (latency 28-30 msec) of the middle latency AEP: Pa amplitudes following binaural stimulation were about 60% larger than those following monaural stimulation. In contrast, long-latency auditory evoked potentials showed total occlusion: N1(95-120 msec) and P2 (170-200 msec) components were as large following monaural as binaural stimulation (see illustration). The results suggest that the breadth of spatial tuning of neurons generating AEPs increases at increasing latencies.

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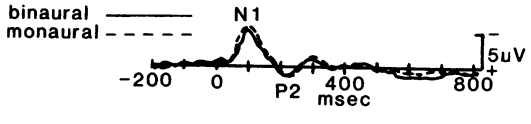

441.14

ULTRASTRUCTURE OF THE BRAINSTEM TIME CODING PATHWAYS IN THE BARN OWL. C. E. Carr and R. E. Boudreau. Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

In the barn owl, sensitivity to interaural time differences arises in nucleus laminaris (NL), the first recipient of binaural input. Eighth nerve afferents synapse upon the neurons of the magnocellular cochlear nucleus (NM), which in turn project bilaterally to NL. The ipsilateral axons from NM enter NL dorsally, while the contralateral axons enter ventrally. The dorso-ventral arrays created by the interdigitation of these afferents form maps of interaural time difference.

At the ultrastructural level, the owl's time coding pathway resembles that of the chick. Each neuron in NM receives two types of synaptic input. One is the calciform ending typical of eighth nerve fibers. These profiles contain round clear vesicles, and form a large number of punctate asymmetric synapses. The other type of profile is smaller and densely packed with ellipsoid vesicles. These profiles may correspond to the GAD positive terminals observed with the light microscope.

The afferent axons from NM form frequent nodes within NL, as well as many large club shaped terminals upon the dendrites and somata of NL neurons. Each NL neuron has a large oval soma, about 30μm in diameter, and short thick dendrites. As in the chick, NL neurons receive thick punctate synaptic densities from NM terminals and a second type of profile which forms narrow single densities (Parks et al., (1983) J. Comp. Neurol. 214:32-42). NL neurons lack an initial segment; the large (10μm) axon becomes myelinated at the soma, and membrane adjacent to the origin of myelination does not contain a dense undercoating.

Supported by NIH grant R29 NS25507.

441.15

HOW IS THE FRONTAL AUDITORY SPACE REPRESENTED IN THE AUDITORY CORTEX OF THE FM BAT, EPTESICUS FUSCUS? P.H.-S. Jen, X.D. Sun* and P.J.-J. Lin*. Div. of Biol. Sci. The Univ. of Missouri, Columbia, MO. 65211.

The spatial sensitivity of single neurons and auditory space representation in the primary auditory cortex of Eptesicus fuscus was studied under free field stimulus conditions. A 4 ms pure tone pulse was delivered from a loudspeaker placed 23 cm in front of the bat to determine the best frequency (BF) and minimum threshold (MT) of each neuron. Then an FM signal sweeping one-octave downward across the neuron's BF was delivered as the loudspeaker was moved through the frontal auditory space to determine the spatial response center at which the neuron's MT was the lowest. The stimulus was then raised 10-20 dB above each neuron's lowest MT to measure the spatial response area. All response centers are located in the contralateral frontal auditory space (0^{o} and 50^{o} in azimuth, 0^{o} and 25^{o} in elevation). Response centers tend to move toward the midline and slightly downward with increasing BF. Since high BF neurons are located anteriorly and low BF neurons posteriorly, the auditory space appears to have an orderly representation in the auditory cortex along the tonotopic axis. Thus, the lateral space is represented posteriorly and the middle space anteriorly. While the response area of cortical neurons expands asymmetrically with stimulus intensity, high BF neurons tend to have smaller spatial response areas than low BF neurons. The BFs, MTs, spatial response areas and response centers of neurons sequentially isolated from an orthogonally penetrated electrode are similar indicating columnar organization in the auditory cortex.

441.16

LOW-FREQUENCY TONE DISCRIMINATION IN THE CLAWED FROG, XENOPUS LAEVIS. A.Elepfandt, M.Hainich*. Fak. Biol., Univ. Konstanz, P.O.Box 5560, D-7750 Konstanz, Fed. Rep. Germany.

Discrimination of tones below 800 Hz, which fall into the frequency range of the amphibian papilla, was investigated in the clawed frog, Xenopus laevis. Because Xenopus is aquatic, this discrimination is of underwater sound.

In the tests, sound was presented from the bottom of a tank 65 cm in diameter filled with water to a depth of 45 cm. The frog moved on a net 7 cm below the water surface. Discrimination between trains of 6 tone pulses of constant or alternating frequency was conditioned by a go/no-go method. FFT analysis showed that the presented tones were monofrequent throughout the test area.

Most frogs learned the discrimination and remembered it for several weeks. Relative discrimination limens (DL) were 0.05-0.1, which coincides with the acuity of tone discrimination in non-mammalian vertebrates. This accuracy is considerably greater than found earlier for frequencies above 1 KHz (DL=0.45), which may reflect the fact that a tonotopic organization is found in the amphibian but not the basilar papilla of frogs.

441.17

AUDITORY DURATION DISCRIMINATION IN THE EUROPEAN STARLING (Sturnus vulgaris). E. H. Maier* and G. M. Klump*. (SPON. P. M. Narins). TU Munich, Inst. of Zoology, Lichtenbergstr. 4, D - 8046 Garching, FRG.

Three starlings were tested in a GO/NOGO-procedure on their ability to detect changes in the duration of a tone. Stimuli were pure tones at three frequencies (4 kHz, 2 kHz, 0.5 kHz) and three standard durations (800 ms, 200 ms, 100 ms). Stimulus amplitude was randomized in steps of 0.375 dB in the range of 48 to 52 dB SPL. Increments and decrements in tone duration ($\pm \triangle$ T) ranged from 5% to 90% of the standard duration. In the range of the presumed threshold they varied in steps of 5% of the standard duration. Stimuli of different durations were presented by the method of constant stimuli. Thresholds were determined using signal detection theory, threshold criterion was the discrimination measure d' of 1.8.

For an increase in duration no influence of frequency on the discrimination threshold was found (n=2). Weber fractions $\triangle$ T/T ranged from 0.12 at 800 ms standard duration to 0.23 at 100 ms standard duration. For the bird tested with a decrease in tone duration neither stimulus frequency nor standard duration had an influence on the thresholds (average $\triangle$ T/T was 0.19). The results of this animal and of one additional bird in which both thresholds for $+\triangle$ T and $-\triangle$ T were measured, suggest that it may be more difficult for the starling to detect a decrease in duration.

(Supported by the Deutsche Forschungsgemeinschaft, SFB 204, Gehör.)

441.18

UNILATERAL NEONATAL DEAFENING RESULTS IN BILATERAL AUDITORY CORTICAL PATHOLOGY. Nathaniel T. McMullen, Bruce Zukerberg* and Edmund M. Glaser. Dept. of Physiology, Univ. of Maryland Sch. of Med. Balt. MD 21201.

In recent papers we have demonstrated that unilateral cochlear damage in the neonatal rabbit results in substantial alterations in presumptive target neurons in the contralateral auditory cortex (McMullen et al, JCN, 267: 92-106, 1988; McMullen & Glaser, Exp. Brain Res. 1988). Based on 3-D reconstruction and quantitative analyses of 100 Golgi-impregnated neurons obtained from 4 neonatally deafened and 2 control rabbits, we now report that spine free nonpyramidal cells ipsilateral to the neonatally damaged cochlea exhibit a nearly identical pattern of dendritic expansion (ca. 24% increase in total length) and abnormally recurved dendrites (42% of cells). The expansion was due entirely to increased branch length as there was no change in the number of dendritic branches. Recurved dendrites were almost exclusively directed tangentially or toward the white matter. These data indicate that unilateral cochlear damage results in the reorganization of both ipsilateral and contralateral ascending auditory pathways. The failure of the undamaged ear to maintain normal cortical dendritic growth bilaterally is evidence that binaural competition is an important component of auditory cortical maturation.

Supported by NIH grants NS17861, RR02169 and the Deafness Research Foundation.

441.19

DEVELOPMENTAL BASIS OF CONGENITAL DEAFNESS WITH CARDIAC ARRYTHMIAS. M.J. Mulroy. Dept. Anatomy, Med. College of Georgia, Augusta, GA 30912.

The long QT syndrome is characterized by abnormal prolongation of the QT interval of the electrocardiogram, and is sometimes associated with congenital deafness. The resulting arrythmias can be life threatening. We have developed an animal model in which to study the changes in the innervation of the heart and inner ear associated with this syndrome.

Nodose and otic placodes were removed from 30-36 hour old chick embryos. ECGs were recorded on embryonic day 17. Cochlear potentials of embryos with long QT intervals were measured to assess the function of the ear. Sensory, parasympathetic and sympathetic innervation of the heart was evaluated. The cochlea was also evaluated histologically.

Data will be presented to support the hypothesis that a small lesion affecting the nodose and otic placodes during early development, when the placode are contiguous, can alter the normal innervation of the heart and cochlea resulting in cardiac arrythmias and deafness.

Supported by Am. Heart Assoc. GA Affiliate.

441.20

INDIVIDUAL NEURONAL AREA CHANGES IN THE DEVELOPING AND AGED MEDIAL NUCLEUS OF THE TRAPEZOID BODY OF HEARING IMPAIRED C57BL/6 MICE. R.H. Browner and E.R. Riedel*. Department of Anatomy, New York Medical College, Valhalla, NY 10595.

Area changes in the cells of the medial nucleus of the trapezoid body were analyzed in Nissl stained brains in 10-, 30-, and 2 year old mice. Anesthetized animals were transcardiaclly perfused with saline and then Susa's fixitive and paraffin embedded. Cross sections of 15um were measured using a F. Haer morphometric quantitative data analysis system. Measurements were made on a 100x oil planoapochromatic olympus objective. At least 2 animals (both nuclei) were measured in each survival stage. Every 4th section was measured throughout the length of the nucleus and at least 15 to 20 neurons were measured in these sections. Mean individual cell area were 10-day 139.80 um^2, 30-day 167.86 um^2, 2 year 142.58 um^2. Using T-test and ANOVA statistical methods there are significant differences between the 10- and 30-day, and 30-day and 2 year old mice; none between 10-day and two year old mice. This indicates a cyclic change in individual cell area which first increases and then decreases. This work was supported by the Culpeper and the Jurzkowski Founds and the NY Acad of Sciences.

442.1

CORTICAL DEAFNESS CANNOT ACCOUNT FOR "SENSORY APHASIA" IN JAPANESE MACAQUES. H. E. Heffner and R. S. Heffner., Dept. of Psychology, Univ. of Toledo, Toledo, OH 43606.

Bilateral ablation of the superior temporal gyrus in Japanese macaques results in a significant hearing loss (cortical deafness) as well as in an inability to discriminate between two types of their "coo" vocalizations ("sensory aphasia"). A two-part investigation was conducted to determine whether the inability to discriminate the vocalizations might simply be the result of the hearing loss itself.

First, four normal Japanese macaques were tested for their ability to discriminate coos which were attenuated to simulate the effect of a cortical hearing loss. Second, four Japanese macaques with bilateral superior temporal gyrus lesions were tested for their ability to discriminate coos which had been amplified and equalized to compensate for each animal's hearing loss.

All four normal macaques were easily able to discriminate the coos which had been attenuated to simulate a cortical hearing loss. However, amplifying the coos to compensate for the operated monkeys' hearing losses did not improve their performances.

It appears that the inability of monkeys with bilateral superior temporal gyrus lesions to discriminate conspecific vocalizations is not simply due to the accompanying hearing loss, but is a separate auditory disorder. (Supported by NIH grant NS 12992)

442.2

HISTOPATHOLOGICAL CHANGES OF THE COCHLEA AND AUDITORY NERVE FOLLOWING IMPLANTATION OF A COCHLEAR ELECTRODE IN A DEAF ANIMAL MODEL. S.A. Larsen. Dept. of Anat. Sci. & Neurobiol., Univ. of Louisville, Louisville, KY, 40292.

Histopathological changes which occur following implantation, presence and stimulation of a cochlear implant are of significant concern. Previous studies have shown that the insertion, physical presence and electrical effects of cochlear prostheses result in damage to the cochlea, auditory nerve and cochlear nuclei. Many of the published reports employed normal hearing animals or those in which the scala tympani was injected with an ototoxic drug at the time of implantation of the intracochlear electrode. In this study, monkeys were deafened using systemically delivered ototoxic drugs. Auditory brainstem responses were used to confirm the degree of deafness after which the animals were either given implants or had sham surgery. Two designs of cochlear electrodes were evaluated for their histopathologic effects. Implanted, sham surgical and deafened animals were evaluated histopathologically with scanning and transmission electron microscopy. Computer-assisted morphometric analysis was performed on cochleas and nerves. No differences in the cochleas or auditory nerves of the implanted, sham surgical and the deaf control animals were detected. These results indicate that deaf and normal-hearing animals respond differently to the presence of cochlear electrodes. Supported by a grant from the Symbion Corporation.

442.3

THE EFFECTS OF OTITIS MEDIA WITH EFFUSION INDUCED BY INOCULATION OF THE GERBIL BULLA WITH NON-VIABLE BACTERIA. M.E. Hutchings* and J.S. Kroll*. (SPON: C. Blakemore). University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, England and University Department of Paediatrics, John Radcliffe Hospital, Oxford, England.

Otitis media with effusion (OME) is the major cause of hearing disorders in children and they may suffer fluctuating auditory deprivation for months prior to surgery. The effects of OME on the auditory brainstem are being studied in the gerbil (Meriones unguiculatus) using Haemophilus influenzae, the primary bacterial pathogen associated with chronic human OME.

The right bullae of anaesthetised gerbils were inoculated with either 30ul pyrogen free saline or saline containing non-viable H. influenzae type b Eagan (1.3 x 10^9/ml). Animals were sacrificed at intervals and the temporal bones decalcified, sectioned and examined. Middle ears inoculated with bacteria were inflamed and contained serous effusions which resolved by 14 days post-operation. The round window was thickened and polymorphonuclear leucocytes were present in the scala tympani by one day post-operation and in the scala vestibuli by three days. Middle ear pathology included the formation of granulation tissue and new bone. The mucoperiosteum remained elevated one month post-operation. Saline inoculated ears developed scant effusions and the tympana were normal by 5 days post-operation.

442.4

ANIMAL MODEL OF TINNITUS: SPECIFICITY OF THE PARADIGM. P.J. Jastreboff, J.F. Brennan, C.T. Sasaki. Sect. ENT, Yale U. Sch. Med., New Haven, CT 06510; Dept. Psychology, UMASS, Boston, MA 02125.

Tinnitus, phantom auditory perception, is a widespread disorder of unknown origin. We proposed an electrophysiological and behavioral model of tinnitus, recently focusing on specificity of the behavioral paradigm. Water deprived adult pigmented rats (36), exposed to continuous noise, received lick training, acclimation to 30 s offsets of background noise used as the conditioned stimulus (CS), suppression training involving termination of CS with mild footshock, and 5 days of extinction. Previously, daily injections of salicylate starting before/after suppression training resulted in contrasting behaviors, consistent with the hypothesis of salicylate-induced tinnitus.

To address an alternative explanation based upon salicylate-induced changes in the perception of background auditory context, the noise was decreased by 20 dB starting before or after training, in place of salicylate injections. This manipulation had no behavioral effect, thus further supporting our tinnitus-based explanation. A second experiment employed 2 doses of quinine (100 and 200 mg/kg) in place of salicylate to induce tinnitus. Quinine injections resulted in changes analogous to those observed following salicylate, in a dose-dependent manner. These data further confirm the specificity of this animal model of tinnitus. (Supported by NIH NS22024 and DRF 1988)

442.5

GENERALIZATION OF CONDITIONED SUPPRESSION IN RATS FOLLOWING SALICYLATE INDUCED TINNITUS. J.F. Brennan and P.J. Jastreboff. Dept. of Psychology, University of Massachusetts at Boston, Boston, MA 02125.

The extent of auditory stimulus control of lick suppression was examined by varying the auditory environment of 36 pigmented rats exposed to daily injections of sodium salicylate, 350 mg/kg, introduced either before or after acquisition training. In successive stages, rats received lick training, acclimation exposure to the conditioned stimulus (CS), suppression acquisition sessions involving 5 onsets of 1-min 10-kHz CSi terminating with a 0.5 s, 1-mA footshock, and 5 extinction tests wherein the CS frequencies varied randomly among 7-, 8-, 9-, 10-, and 11-kHz (all at 60-dB). In Experiment I, 18 subjects were exposed continuously to a 7-kHz tone superimposed upon noise of 60-dB, except for the CS probes when only the respective tonal value occurred. In Experiment II, 18 rats were exposed to the 7-kHz background only. Stimulus control was evident in the saline injected control groups of both experiments by the sharp gradients of auditory generalization of suppression obtained within individual subjects. Both groups injected after training showed little auditory control and rapid extinction of suppression. The groups injected before training showed severe suppression and distorted gradients reflecting a summation effect. These data are readily accommodated within a model that infers the differential presence of tinnitus.
(Supported by DRF, 1988)

442.6

REDUCED SUSCEPTIBILITY OF THE COCHLEA TO REPEATED EXPOSURE. BL Lonsbury-Martin, DJ Franklin*, BB Stagner* and GK Martin*. Dept. Otorhinolaryngol. Communicat. Sci., Baylor Col. of Med., Houston, TX 77030.

Susceptibility of the cochlea to repeated exposure was investigated in 4 rabbits with measures of behavioral thresholds and distortion-product emissions (DPEs) from outer hair cells (OHCs). Baseline behavioral thresholds were measured at 11 frequencies (.3-22.6 kHz) representative of rabbit audibility using a conditioning task. DPEs at 2f1-f2 were measured as "audiograms" (1.5-18 kHz, 200 Hz intervals) generated by equilevel primaries (45,55,65 dB SPL) and input-output functions (5-dB steps, 9 frequencies). Following control measures, rabbits were noise-exposed (1-kHz 95-dB SPL octaveband) until a criterion reduction was detected in DPE levels generated by 45-dB primaries from 1-4 kHz. During 3 wks of recovery, DPEs were monitored at regular intervals (1,2,3,7,21 days) and compared to corresponding behavioral measures. By 3-wks postexposure, all rabbits had recovered to baseline behavioral and physiological levels. The exposure-recovery regimen was continued until a permanent 10-dB decrement in DPE level resulted for any frequency from 1-10 kHz. Using this criterion, one rabbit was exposed 2 times while each of the 3 remaining animals received 3 exposures. Following the final recovery, functional measures were evaluated at 5 wks postexposure and cochleas assessed. The major outcome was that each rabbit demonstrated an increased resistance to noise-induced dysfunction. Altered susceptibility was expressed for each successive exposure as a systematic increase in the time required to reach the criterion loss. The notion that hair cells can be "conditioned" to become less sensitive to overstimulation through repeated exposure is a relatively new concept. However, given recent evidence that the OHC system contributes dynamically to stimulus transduction, it is likely that the peripheral efferent system participates in the observed increased resistance to repeated exposure. [NS10940, ES03500, DRF]

442.7

EFFECTS OF BILATERAL ABLATION OF THE AUDITORY CORTEX AND/OR CINGULATE CORTEX ON THE BIOSONAR BEHAVIOR OF THE MUSTACHED BAT. S.J.Gaioni*, N.Suga, and H.Riquimaroux. Dept. of Biology, Washington Univ., St.Louis, MO 63130.

The mustached bat (Pteronotus parnellii) adjusts its complex biosonar signal (pulse) during orientation and insect capture: 1) it lowers pulse frequency below its resting frequency (RF) to compensate for Doppler shift in returning echoes (DSC); 2) it decreases pulse intensity as echo intensity increases; 3) it lengthens pulse CF duration when it detects a flying insect; 4) it then decreases pulse duration and increases pulse repetition rate. The mustached bat's auditory cortex (AC) has several functional subdivisions for processing different types of biosonar information. Further, its highest vocalization center, the cingulate cortex (Cg), contains a motor map representing pulse frequencies emitted during Doppler-shift compensation (Gooler & O'Neill, 1987). We conducted ablation experiments to examine the relationship between these structures and these biosonar behaviors. Quantitative measurements of the behavioral adjustments were made by placing each bat on a pendulum which was swung towards a large target. The bats were then retested following large bilateral ablations by aspiration of the Cg , Cg followed by AC, AC, or DSCF area (a part of the AC). The Cg ablations had no measurable effect on any of these behaviors. In contrast, following AC ablation, the amount and stability of DSC significantly decreased, and reaction time increased. These bats' RFs became more variable post-ablation, and the RFs also showed a large transient increase immediately following each pendulum test session. The DSCF-ablated bats showed changes in DSC similar to those displayed by the AC-ablated bats. In conclusion: 1) the AC is involved in stabilization of the RF, and its DSCF subdivision is involved in "fine-tuning" of DSC; 2) the role of the Cg in biosonar behavior is enigmatic. (Supported by AFOSR grant #86-NL-192.)

442.8

ARE THE MECHANISMS OF DELAY-DEPENDENT INTEGRATORS FOR RANGING DIFFERENT IN TWO SPECIES OF BATS, LITTLE BROWN BAT AND MUSTACHED BAT? A. Berkowitz* and N. Suga. Dept. of Biology, Washington Univ., St. Louis, MO 63130.

For echolocation, the little brown bat, *Myotis lucifugus*, emits FM pulses with no harmonics. In the auditory cortex, many neurons show a facilitated response selectively to an artificial pulse-echo pair with a particular delay between them. Many of these respond at short latency to a single weak pulse and at longer latency to a strong pulse. This paradoxical latency shift could be responsible for delay-dependent facilitation. In contrast, the mustached bat, *Pteronotus parnellii*, emits CF-FM pulses with 4 harmonics. In the auditory cortex, neurons also show delay-dependent facilitation, but each responds selectively to a pulse at the fundamental paired with an echo at one of the higher harmonics. These neurons utilize delay lines created by sub-thalamic neurons tuned to the fundamental. This study investigates whether delay-dependent integration in *Myotis* occurs through a different mechanism. Using tungsten microelectrodes on unanesthetized animals, the relationship between delay-dependent facilitation and paradoxical latency shift was examined in cortical auditory neurons. For those neurons which showed both phenomena, the magnitude of the latency shift was highly correlated with the best delay for facilitation. This suggests that pulse and echo are distinguished by intensity and that superimposition of pulse and echo inputs onto integrator neurons underlies determination of target distance in *Myotis*. Our data also suggest that high intensity pulses evoke inhibition whereas low intensity pulses do not, and this may underly paradoxical latency shift. We conclude that *Myotis* delay-dependent integrators make use of paradoxical latency shift, a mechanism different from that employed for the same function in *Pteronotus*. (Supported by NIH research grant NS17333.)

442.9

ICV INJECTION OF CARBACHOL SUPPRESSES ACOUSTIC STARTLE RELATED EMG-AMPLITUDE. M.Th. Kaltwasser*, H.U.Schnitzler* (SPON: EBBS). Dept. of Animal Physiology, Univ. of Tuebingen, Morgenstelle 28, 7400 Tuebingen, Fed. Rep. Germany.

The acoustic startle response is a simple behavioral model for studying the neural mechanism underlying the transformation of a sensory input to a motor output. Several transmitters were found to modulate this short latency reflex. Since systemic injection of acetylcholine (Ach) antagonists enhances the startle response, and since an early relay station in the startle circuit, the cochlear nucleus, is cholinergic, the present study investigated the role of Ach in modulating the startle response at the cerebral level. Microinjection of 500ng carbachol (Ach agonist) into the lateral ventricle yielded a significant suppression of the startle related EMG-amplitude of the temporalis muscle. ICV injection of 500ng atropin (Ach antagonist) showed a slight tendency for enhancing the EMG-amplitude. These results suggest that Ach modulates the motor response to a high intensity acoustic stimulus. Preliminary data indicate that the last relay station in the startle circuit, the motor trigeminal nucleus, is not the target site for this cholinergic action.

442.10

TECTAL MODULATION OF ACOUSTIC STARTLE IN THE RAT. D. S. Leitner and M. E. Cohen. Psychology Dept., Saint Joseph's Univ., Philadelphia, PA 19131 & Dept. of Psychology, Bryn Mawr Col., Bryn Mawr, PA 19010.

Sensory events which do not themselves elicit startle (prestimuli) can alter the latency or amplitude of startle depending upon the amount of time by which the prestimulus precedes the startle-eliciting stimulus. Previous work has demonstrated that the inferior colliculus (IC) plays a role in the reduction of acoustic startle amplitude in the rat by auditory prestimuli. The present study investigated the role played by the superior colliculus (SC), a visual structure, in startle amplitude reduction.

Eighteen rats were pretested for acoustic startle amplitude reduction using auditory and visual prestimuli. Seven rats were then given SC lesions, 5 were given IC lesions, and 6 served as unoperated controls. All subjects were then posttested in a manner identical to the pretest.

Data analysis showed the SC group had a reliable pre- to posttest decrease in percent startle amplitude reduction produced by the visual prestimulus but no change in that produced by the auditory prestimulus, the IC group showed a significant decrease in amplitude reduction produced by the auditory prestimulus but not the visual prestimulus, and no changes were present in the amplitude reduction produced by either prestimulus in the control group. The SC group showed reliable latency reduction to the visual prestimulus when the interstimulus interval was reduced, indicating that they were still capable of processing visual stimuli. These data replicate and extend previous findings by demonstrating that the SC plays a prominent role in amplitude reduction by visual prestimuli.

MOTIVATION AND EMOTION I

443.1

MICRODIALYSIS SHOWS INCREASED DOPAMINE TURNOVER IN THE NUCLEUS ACCUMBENS DURING LATERAL HYPOTHALAMIC SELF-STIMULATION. G. A. Hunter, L. Hernandez and B. G. Hoebel. Dept. Psychol., Princeton Univ., Princeton, NJ 08544

Extracellular dopamine (DA), and its major metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured in five rats trained to lever press for a fixed current of perifornical lateral hypothalamic (PFH) stimulation. A microdialysis probe was inserted into the posterior accumbens through a previously implanted guide cannula ipsilateral to the stimulation electrode, and a series of baseline samples was taken at 20 min intervals for analysis by high pressure liquid chromatography with electrochemical detection. After DA levels had stabilized, the animals were allowed to self-stimulate for 1 hr while sampling continued. Response rates were between 2400 and 3000/hr. Extracellular levels of DOPAC and HVA increased significantly, indicating an increase in DA turnover that persisted beyond the period of self-stimulation by about 40 min. Extracellular dopamine also tended to increase. These results demonstrate an increase in DA turnover in the accumbens associated with self-stimulation in freely moving rats. Stimulation of the PFH that induces feeding has also been shown to increase extracellular DA, DOPAC and HVA in another study (1) which suggests that DA turnover in the accumbens is involved both in the reinforcement of PFH self-stimulation and the induction of food intake.

1. Hernandez, L. & Hoebel, B. G. Life Sci., 1988, 42, 1705-1712.

443.2

DOUBLE-LABEL DEOXYGLUCOSE AUTORADIOGRAPHIC STUDIES OF UNILATERAL AND BILATERAL MEDIAL FOREBRAIN BUNDLE SELF-STIMULATION IN RATS. R.F. Ackermann and M.E. Phelps. Division of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, CA 90024.

We are studying intracranial self-stimulation (ICSS) responding with [F-18] fluorodeoxyglucose (FDG) and [C-14] 2-deoxyglucose (2DG) double-label autoradiography in rats having chronic bilateral medial forebrain bundle (MFB) electrodes. Double-label autoradiography allows visualization of two conditions in each animal: control vs. stimulation; left stimulation vs. right stimulation; and unilateral stimulation vs. simultaneous stimulation of both left and right electrodes. The FDG/2DG data obtained thus far have confirmed that unilateral MFB stimulation produces bilateral increased-utilization patterns (cf. Porrino et al., Science, 224:306-309, 1984); simultaneous stimulation of both MFB sites enhances the already bilateralized single-stimulation utilization patterns. These patterns comprised two areas not specifically noted in previous 2DG/ICSS studies: the dorsolateral mesencephalic tegmentum, and the cerebellum. Utilization in these two structures strongly resembled that reported for motor cortex stimulation (Sharp and Evans, J. Comp. Neurol., 208: 255-287, 1982). The cerebellum itself is an ICSS site (Ball et al., Physiol. Behav., 13:123-127, 1974), and may play a greater role in integrating the sensory-motor components of ICSS behavior than previously suspected (cf. Sharp and Gonzalez, J. Comp. Neurol., 234:498-500, 1985).

443.3

TWO PROPERTIES OF THE INTEGRATOR FOR REWARDING BRAIN STIMULATION. G. Fouriezos, S. Walker* and K. Paterson*. School of Psychology, University of Ottawa, Ottawa, Canada. K1N 6N5.

Four experiments evaluated the effect on self-stimulation frequency thresholds of altering baseline activity of the directly stimulated reward substrate. Baseline activity was altered by applying a continuous, low frequency of stimulation pulses while the rats pressed a lever to earn bursts of lateral hypothalamic stimulation at the same time. Continuous stimulation (0, 2, 5, 10 or 20 Hz) decreased frequency thresholds by its own frequency when it was applied to the same electrode (Expt 1) and by frequencies predicted by empirically determined summation levels when it was applied to a contralateral electrode (Expts 1 & 2). The effect was additive over a wide range of base frequency thresholds (Expt 3) but it was completely eliminated when the continuous stimulation was restricted to times between response-triggered bursts (Expt 4). We conclude, first, that the reward integrator defends an absolute threshold in the sense that it is not sensitive to baseline activity and, second, that the integrator accepts input only for the duration of the response initiated train.

443.4

INVOLVEMENT OF DOPAMINE D2 RECEPTORS IN THE REINFORCEMENT OF OPERANT BEHAVIOUR S. Nakajima Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada

Previously I have reported that a dopamine D1 receptors are involved in the neural mechanism of reinforcement. Now the effect of dopamine D2 receptor blockade on operant behaviour was examined using raclopride.

In the rats trained to press a bar to obtain food, injection (IP) of raclopride suppressed responding in a dose related manner. Another group of rats were trained to press a bar for electrical stimulation of the ventral tegmental area. Raclopride again suppressed responding in a dose related manner. Compared with food-reinforced responses, about 10 times smaller dose was sufficient to suppress self-stimulation. Responding for stimulation at 160 Hz was more easily suppressed than responding for 60 Hz. Injection of raclopride into the nucleus accumbens suppressed self-stimulation of the ipsilateral ventral tegmental area (ED 50 = 10μg).

Blockade of dopamine D2 receptors seems to interfere with the brain mechanism of reinforcement. (Raclopride was a gift of Astra Alab AB, Sweden)

443.5

EFFECTS OF ANTERIOR MEDIAL FOREBRAIN BUNDLE LESIONS ON SELF-STIMULATION OF THE LATERAL HYPOTHALAMUS AND VENTRAL TEGMENTAL AREA. B. Murray and P. Shizgal. CSBN, Concordia University, Montreal, Quebec H3G 1M8.

Psychophysical data suggest that reward fibers directly link the lateral hypothalamus (LH) and ventral tegmental area (VTA). As a step toward identifying the nuclei from which these fibers arise, we assessed the effect on self-stimulation of both the LH and VTA of electrolytically lesioning the anterior medial forebrain bundle (MFB). Changes in the rewarding effect were inferred from lateral displacements of rate-frequency functions. Lesions in 5 of the 7 rats displaced the rate-frequency functions for the LH and/or VTA sites toward higher frequencies (26-37% above baseline), an effect consistent with a decrease in the rewarding impact of the stimulation. The ineffective lesions were restricted to compartment 'c' of the anterior MFB (Nieuwenhuys et al. J. comp. Neurol., 1982, 206, 49) while the five effective lesions invaded the more lateral compartments, 'a', 'd', and 'e'. Thus, the shifts in the rate-frequency functions may have been caused by damage to neurons projecting through compartments 'a', 'd', and 'e', or neurons with somata in the lesioned areas of the antero-lateral MFB. Although these shifts could also have resulted from damage to ascending dopaminergic fibers, it is not clear that the distribution of these fibers is consistent with the differential effect of the medial and lateral lesions.

443.6

MODULATION OF COLLISION EFFECTS BY LESIONS: IMPLICATIONS FOR IDENTIFYING NEURONS SUBSERVING BRAIN STIMULATION REWARD. P. Shizgal and B. Murray. CSBN, Concordia University, Montreal, Quebec H3G 1M8.

Lesion methods have long been used to identify the neurons responsible for the rewarding effects of electrical brain stimulation. Unfortunately, the anatomical interpretation of such data is ambiguous. A decrease in the rewarding effect could be due to damage to the directly stimulated neurons, their efferents, or neurons that modulate transmission in the reward circuit. In contrast, a collision effect inferred from psychophysical data is consistent with a clear anatomical interpretation: The tips of the two stimulation electrodes lie along the axonal trajectories of a common population of reward neurons. By combining collision tests with lesions, we hoped to render the anatomical interpretation of lesion data less ambiguous. We reasoned that if a lesion destroyed some of the reward neurons undergoing collision, then the collision effect would decrease in size.

Trains of pulse pairs were applied to the LH and VTA, with each electrode delivering either the conditioning (C) or the test (T) pulses. Collision was inferred from an abrupt decrease in stimulation effectiveness as the C-T interval was decreased. In 2 of the 4 rats, a lesion aimed 1.5 mm anterior to the LH site decreased the collision effect by 25% to 30%. The simplest interpretation of these data is that the lesions damaged reward fibers directly linking the two sites.

443.7

SMALL LESIONS IN THE ANTERIOR VENTRAL TEGMENTAL AREA (VTA) ATTENUATE THE REWARDING EFFICACY OF LATERAL HYPOTHALAMIC (LH) SELF-STIMULATION. Paul W. Glimcher and C. R. Gallistel. Department of Psychology and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Male rats (350g) were implanted with monopolar stimulating electrodes in the LH (A/P -2.2 to bregma; M/L 1.5 ;D/V 8.3 to dura) and in the anterior VTA (-4.6;0.7;7.8). Animals were trained to lever press for 0.5sec trains of 0.1msec cathodal pulses at both electrodes. The frequencies of these trains were systematically varied allowing identification of the frequency of stimulation which produced half maximal rates of lever pressing at a number of currents for each electrode. Small electrolytic lesions (300uA/20sec) were made at the VTA electrode. Frequencies necessary to produce half maximal responding at the same currents were again characterized. Animals showed post-lesion shifts in the efficacy of rewarding stimulation at the LH electrode as large as 0.9 log units (as measured by the rate/frequency function (Gallistel et al. *Psych Revs.* 88, 228-273). This work, considered in light of Bielajew & Shizgal's results (*J Neurosci.* 6, 919-929) suggests that a large portion of the reinforcing signal generated by the LH electrode passes through the VTA lesion site.

This work was supported by Grant NSF BNS86-19759.

443.8

SELF-STIMULATION AND ELECTRICALLY INDUCED LOCOMOTOR ACTIVATION IN THE LATERAL HYPOTHALAMUS OF THE RAT ARE MEDIATED BY DISTINCT NEURONAL SUBSTRATES. L. Velley, C. Verney*, E.Kempf* and B. Berger*. Lab. Psychophysiologie, Univ. Bordeaux I, Avenue des Facultés 33405 Talence Cedex France.

The respective roles of intrinsic neurons and of catecholaminergic fibers in two behaviors (self-stimulation and the increase of locomotion produced by non-contingent stimulation) elicited by electrical stimulation of the lateral hypothalamus (LH), were tested. Groups of rats were injected in one LH with either ibotenic acid (4 μg/0.5 μl), 6-hydroxydopamine (2 μg/0.5 μl) or with the vehicle of each neurotoxin. Eight days later all rats were bilaterally implanted with stimulation electrodes, one in the lesioned area, the other in the contralateral LH. Whatever the lesion or the behavior tested, the response of the unlesioned LH was similar to that of vehicle-injected rats. Self-stimulation was disturbed in the ibotenic acid lesioned LH but was not modified in the 6-hydroxydopamine treated LH despite losses of catecholamines in the hippocampus and in the striatum and despite a total disruption of the catecholaminergic fibers in the lesioned area. Conversely, the locomotor increase elicited by non-contingent stimulation, measured in the open-field, was normal in the LH lesioned by ibotenic acid, but was not present following stimulation of the 6-hydroxydopamine treated LH, thus indicating distinct neuronal substrates for the expression of these two behaviors.

443.9

EXCITOTOXIN LESIONS OF LATERAL HYPOTHALAMUS FAIL TO DISRUPT SELF-STIMULATION REWARD.
J.R. Stellar, M. Waraczynski, and F.S. Hall.* Psychology Dept., Northeastern University, Boston, MA 02115

Last year, we reported (Waraczynski & Stellar Neurosci.Absts.,13:1324,1987), that microinjections of the neuronal cell body excitotoxin, ibotenic acid, into the lateral hypothalamus (LH) also may produce bleaching on Weil stain, suggestive of axon demyelination. Because demyelination impairs the effectiveness of brain stimulation in recruiting axons, studies (e.g. cited in above ref.) showing depressed LH stimulation reward after excitotoxin may have exaggerated the role of indigenous cells in LH reward.

This year, we report results of ibotenic acid and NMDA lesions of LH in 23 rats, where microinjections were placed so that the area of demyelination was away from the electrode tip, but the area of neuronal cell loss enveloped it. LH reward was assessed with a standard rate-frequency paradigm(Behav.Neur., 101:832,1987). Lesions produced no-to-small (>0.1 Log Hz) decreases in LH reward unless the demyelination zone reached the electrode tip.

Supported by WhiteHall Foundation grant to J.S.

443.10

EFFECTS OF NALOXONE ON REWARD INDUCED BY DORSAL RAPHE ELECTRICAL STIMULATION: A STUDY USING THE CURVE-SHIFT PARADIGM. B.A. Robinson* and P.P. Rompré (SPON: S. Amir). Department of Psychology, Concordia University, Montreal, Quebec, Canada H3G 1M8.

The present experiment examined the effects of several doses of naloxone on bar-pressing for dorsal raphe electrical stimulation. After stabilization of self-stimulation, rats were tested on different days with vehicle-saline and three doses (2, 4 and 8 mg/kg) of naloxone administered in either an ascending or descending order of dosage. Curves relating bar-pressing rates to the stimulation frequency were obtained five times just before drug injection, and repeatedly 0 to 90 min after drug injection. Naloxone shifted the curve to the right, increasing frequency thresholds for dorsal raphe stimulation. The increase in threshold was not dose-dependent, the largest shift was observed (0.15 log unit) at 4 mg/kg, 60 to 90 min after the injection. Because naloxone reduced the maximal response rates by less than 10%, we inferred that the shift in frequency threshold was due to a decrease in the rewarding effectiveness of the stimulation. No significant difference was found between shifts in threshold obtained when the drug was administered in an ascending or a descending order of dosage. These results show that like rewarding stimulation of the medial forebrain bundle, rewarding stimulation of the dorsal raphe is inhibited by blockade of opiate receptors.

443.11

NUCLEUS ACCUMBENS OPIOIDS FACILITATE BRAIN STIMULATION REWARD. T.E.G. West* and R.A. Wise. Psychol. Dept., Concordia University, Montreal, Canada H3G 1M8.

The rewarding effect of intravenous opiates and lateral hypothalamic brain stimulation are both thought to depend at least in part on activation of the mesolimbic dopamine system. Opioids have rewarding actions both near the dopamine cell bodies and near the dopamine terminals, suggesting two sites of entry into the reward circuitry. However, morphine is reported to facilitate the rewarding effects of lateral hypothalamic stimulation when injected near the dopamine cell bodies but not near the dopamine terminals. That nucleus accumbens (NAS) opiates should be rewarding in their own right but should not potentiate rewarding hypothalamic stimulation seems paradoxical, and the present study further explored the effects of NAS microinjections of morphine (5, 10 & 20 ug) and the selective mu agonist DAGO (50, 200, & 800 ng). Each opioid had robust effects. Morphine decreased BSR threshold, shifting the rate-frequency function to 0.12 log units to the left with no change in asymptote. DAGO produced similar effects. The effects of both opioids were blocked by naloxone (1 mg/kg), indicating that the effects of the central opioid injections were receptor-mediated. These data thus confirm reward-potentiating actions of opiates in the region of dopamine terminals in nucleus accumbens; however, the potency of opioids in NAS seems less than that reported for ventral tegmental morphine injections.

443.12

DEPOLARIZATION INACTIVATION OF A9 OR A10 DOPAMINE NEURONS BLOCKS BAR-PRESSING FOR ELECTRICAL STIMULATION OF THE MEDIAL FOREBRAIN BUNDLE. P.P. Rompré, T. West*, S. McGaraughty* and R.A. Wise. Department of Psychology, Concordia University, Montreal, Quebec, Canada H3G 1M8.

Previous experiments have shown that systemic pimozide and ventral tegmental area (VTA) morphine can induce dopamine depolarization inactivation; this results in a complete suppression of bar-pressing for central gray brain stimulation reward (BSR). We now report the effects of systemic pimozide and morphine injections in either VTA or substantia nigra (SN), on medial forebrain bundle BSR. Estimates of BSR threshold were obtained using the curve-shift paradigm under different drug treatments. At low doses (2.5-5 ug), morphine in either the SN or VTA induced a significant decrease in BSR threshold. Higher doses (5-10 ug) of morphine given after a systemic injection of pimozide completely blocked bar-pressing. The behavior was reinstated with either central injection of muscimol (12.5-25 ng) or systemic baclofen (0.5 mg/kg). Because similar treatment with GABA agonists is known to reverse dopamine depolarization inactivation, we infer that blockade of bar-pressing after systemic pimozide and central morphine was due to blockade of dopamine cell firing.

443.13

SCH 23390 SHIFTS THE SELF-STIMULATION RATE-FREQUENCY FUNCTION BY 0.6 LOG UNITS. R.A. Wise and P.P. Rompré (SPON: A.A. Giovino). Psychol. Dept., Concordia University Montreal, Canada H3G 1M8.

D-2 dopamine antagonists block post-synaptic dopamine receptors and can cause feedback-mediated depolarization inactivation of dopaminergic cells. To determine if such inactivation might account for the precipitous nature of extinction of responding for brain stimulation reward, we assessed the effects of the D-1 antagonist SCH 23390, which blocks the effects of post-synaptic dopamine agonists with little or no effect on dopamine feedback mechanisms. Rats were trained to bar press for MFB stimulation and were tested on different days with ascending doses of SCH 23390 (0.01 to 0.32 mg/kg). At the highest dose tested, SCH 23390 shifted rate-frequency functions by 0.6 log units before lever-pressing failed completely; this is twice the maximum shift seen with the D-2 antagonist pimozide. The more graded attenuation of response under SCH 23390 is consistent with the hypothesis that the precipitous failure of self-stimulation under pimozide results from the combination of post-synaptic dopaminergic blockade with a failure of effective transmission in the dopaminergic link in the reward pathway--such a failure of transmission has been demonstrated electrophysiologically with several D-2 antagonists.

443.14

CHRONIC NEUROLEPTIC TOLERANCE AND SENSITIZATION IN THE CURVE SHIFT BRAIN STIMULATION REWARD PARADIGM. M.R.Lynch and R.J.Carey, VA Med Ctr & SUNY HSC, Syracuse, NY 13210

Haloperidol (Hal) induced catalepsy shows a tolerance with chronic treatment rather than sensitization which would be predicted by depolarization block hypotheses of delayed onset clinical effects. The present study was conducted to examine tolerance vs sensitization of motor vs "reward" effects in the curve shift paradigm. Acute 0.07 mg/kg Hal produced reliable increases in threshold for 100 hz biphasic square wave stimulation (0.1sec pulses, 0.1sec interval, 0.2sec train) to the VTA (platinum electrodes). Stimulation was begun at maximum intensity and decreased by 50μA every 5 min, with a 2-min time-out. N=4 rats received Hal 1 hr before testing (pre) for 26 days; n=4 received Hal post and n=4 vehicle pre. The latter two showed consistent rate-intensity functions over test days. The pre group showed tolerance to the threshold increasing effect but there is a suggestion of sensitization for rate suppressing effects in the asymptotic range. Reversing pre and post conditions argued against environmentally-specific tolerance. HPLC-EC of DA and its metabolites on chronic injection days 36-37 revealed partial tolerance to acute Hal-induced stimulation of DA turnover in both pre and post drug groups. Tolerance was evident from DOPAC/DA and HVA/DA ratios but not 3MT/DA. These findings question the relevance of threshold shifts for antipsychotic efficacy and emphasize the need for assessing mechanisms of chronic adaptation processes for different behavioral effects.

443.15

MOTIVATIONAL EFFECTS OF NEUROLEPTICS. H. M. Geyer III*, M. Cornfeldt, V. Ramirez*, R. Corbett* and S. Fielding. (Spon.: S. Puri). Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

A progressive fixed ratio (FR) schedule of milk reinforcement for nose-poke responses was used to assess the effects of neuroleptics. It was hypothesized that motivational deficits would appear as the FR increased and motor deficits would be present across all FR's.

The rats on a restricted diet were pretreated with various compounds, placed in chambers and their nose-pokes for dippers of milk (0.02 ml) were recorded. The performance was not reduced by diazepam at 5 mg/kg or imipramine at 20 mg/kg. However, haloperidol at 0.03 mg/kg, thioridazine at 10 mg/kg, Sch 23390 at 0.005 mg/kg and chlorpromazine at 0.31 mg/kg all reduced responding. The increase in maximum FR's from 12 to 24 and 48 did not effect the performance of saline treated rats. However, chlorpromazine at 0.31 mg/kg reduced the responding at FR 48 but not at FR 12, consistant with a motivational deficit and not motor impairment. Other neuroleptics have shown similar FR dependent decrements in performance, consistant with the clinical reports of neuroleptic induced "anhedonia". The test appears to provide a method for measurement of motivational changes induced by pharmacological agents.

443.16

AMPHETAMINE, APOMORPHINE, AND CHOLECYSTOKININ MODULATION OF DOPAMINE SYSTEMS AND LATERAL HYPOTHALAMIC REWARD F.S. Hall*, J.R. Stellar, M Rice*, C. Meyers* and S. Coffey* (SPON: E. Stellar). Psychol. Dept., Northeastern Univ., Boston, MA, 02115

Dopamine (DA) is accepted as playing an important role in lateral hypothalamic (LH) self-stimulation reward, but it is unclear if DA carries or modulates this reward signal.

Using the standard rate-frequency paradigm, we have confirmed that amphetamine (indirect agonist) increases LH reward in rats and that apomorphine (direct agonist) decreases LH reward. However, the argument that apomorphine disrupts normal DA LH reward signalling by producing non-contingent DA reward signals is not supported by our recent pilot work indicating that apomorphine microinjections into the nucleus accumbens increase LH reward.

In a still more fine-grained analysis based on the rate-frequency paradigm, Cholecystokinin (CCK) microinjection in medial accumbens may increase or decrease LH reward and decrease operant motor capacity, depending on anterior/posterior microinjection locus and on CCK dose (range, 1ng - 10ug).

Supported by WhiteHall Foundation grant to J.S.

443.17

HYPOTHALAMICALLY ELICITED FEEDING AND SELF-STIMULATION: TWO MODES OF INTEGRATION OF THE SAME AXONAL OUTPUT. M. Waraczynski and J.M. Kaplan*. Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104

Many perifornical hypothalamic electrodes that support self-stimulation also elicit feeding. Refractory period and conduction velocity estimates for the axons supporting these behaviors appear identical (Gratton and Wise, Abst. Soc. Neurosci., 11:1176, 1986), suggesting the electrode activates a common first-stage substrate.

The present experiment generates stimulation pulse frequency-response curves for a range of current intensities for self-stimulation and for elicited feeding, using bar press rate and ingestion rate, respectively, as dependent measures. For self-stimulation, increases in intensity shift the frequency-response curves to the left without changing asymptotic performance level. For elicited feeding, increases in intensity elevate the asymptotic ingestion rate, but do not appreciably change the location of the curve along the frequency axis. The qualitatively different parametric profiles obtained uncover radically different integrative processes, acting on output from a common substrate, mediating the respective behaviors.

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443.18

REWARD VERSUS PERFORMANCE EFFECTS OF INSULIN AND 2 DEOXY-D-GLUCOSE ON SELF-STIMULATION. S.A. Frutiger, Psychology Department, University of Wisconsin-River Falls, River Falls, WI 54022.

In a previous study (Frutiger, 1986) insulin and 2 deoxy-D-glucose (2DG) were noted to increase latency to initiate a self-stimulation bout and to decrease the rate of self-stimulation. Since mild activity suppression was noted during periods of noncontingent stimulation at the same lateral hypothalamic sites, these changes in latency and rate cannot be unambiguously attributed to a decrease in the rewarding effect of stimulation. This study was designed to provide a more direct dissociation of changes in self-stimulation due to reward versus performance following insulin or 2DG injections.

Anesthetized male Long-Evans rats were implanted with bilateral lateral hypothalamic electrodes, tested for stimulation-bound eating and/or drinking and self-stimulation, and trained on a fixed interval 8 sec schedule of reward. A stable rate-pulse frequency function was established for each rat at a current intensity which had sustained 50% of asymptotic response rate with a 100 pulse/sec frequency and insulin (2U/kg, sc) and 2DG (200 mg/kg, ip) were tested in counterbalanced order. Drug effects on the locus of rise and asymptotic response rate will be evaluated for the first and second half of the 8 sec inter-reinforcement interval.

MOTIVATION AND EMOTION II

444.1

THE INCENTIVE MOTIVATIONAL PROPERTIES OF FOOD AND OPIATES ARE MEDIATED BY A COMMON BRAINSTEM SUBSTRATE. A.Bechara and D.van der Kooy. Neurobiology Research Group, Anatomy Dept., Univ. of Toronto,Toronto,Canada M5S 1A8.

The reinforcing effects of opiates are dependent on both their incentive and drive reduction properties. Bilateral ibotenic acid lesions(.2 *ul* of a 4 % solution on each side) of the tegmental pedunculopontine nucleus (TPP) abolished the morphine (2-10 mg/kg s.c.) conditioned place preferences produced in opiate naive but not in opiate dependent (60 mg/kg /day for 14 days) rats. These results suggest that the TPP mediates specifically the incentive motivational properties of opiates. Moreover, the incentive motivational and aversive withdrawl properties of opiates can be measured separately by pairing a novel place only with morphine in naive rats and only with morphine withdrawl in separate dependent rats. TPP lesions blocked the place preferences for the morphine paired environment, but not the place aversions to the withdrawl paired environment.

A similar set of experiments was carried out with food (Purina lab chow) rather than morphine as the reinforcer. TPP lesions did not block the food place preferences in food deprived (23 hours) rats, nor did they block the place aversions in food deprived animals conditioned to a place paired with the lack of food. However, TPP lesions did block the place preferences produced by food in sated rats. These sated rats (TPP lesions and sham controls) did not eat the food in the training environment , but controls still showed large preferences for the place paired with the uneaten food. We suggest that reinforcers can act through two parallel motivational mechanisms in the nervous system- a circuit through the TPP that mediates the impact of incentive stimuli, and an independent drive reduction mechanism processing homeostatic signals arising from within the organism.

444.2

AVERSIVE PROPERTIES OF OPIATE RECEPTOR BLOCKADE : EXCLUSIVELY CENTRAL MEDIATION IN NAIVE AND MORPHINE-DEPENDENT RATS. T.H. HAND, G.F. KOOB, L. STINUS and M. LE MOAL. INSERM U.259 - Université de Bordeaux II, Rue Camille Saint-Saëns, 33077 Bordeaux Cedex - France. and BCR1 - Scripps Clinic, La Jolla, CA 92037, USA

The motivational effects of exclusively peripheral or central opiate receptor blockade were studied using place conditioning. Intraventricular (ICV) methylnaloxonium (MN) produced place aversions in both naive (200-1000 ng) and morphine-dependent rats (50-500 ng). Interestingly, the full withdrawal syndrome ("wet dog shakes", jumping, hyperreactivity, teeth chattering, writhing, diarrhea, weight loss) was never observed, although some of these signs were occasionally seen in the dependent group at highest dose. Subcutaneous MN (0.03-10 mg/kg) was ineffective in naive rats and produced place aversions in dependent rats only at the highest dose (a dose at which some central blockade may have occured). These data suggest that the aversive properties of opiate receptor antagonism are centrally mediated in both naive and dependent rats, and that their enhancement in dependent rats results from a sensitized central mechanism rather than from the recruitment of a peripheral component. They also suggest that the so-called "withdrawal syndrome" may not be a suitable model for these aversive effects.

444.3

ESB OF LATERAL HYPOTHALAMIC FEAR/FLIGHT SITES IN RATS PRODUCES CONDITIONED PLACE AVOIDANCE. D.S. Sacks* and J. Panksepp. (SPON: K.F. Green) Dept. of Psych., Bowling Green State University, Bowling Green, OH., 43403.

Electrical stimulation of the brain (ESB) in the anterior lateral hypothalamic area (AHA) has been shown to produce behaviors indicative of emotional states (Behav. Brain Sci., 1982, 5:407). Stimulation-induced "fear" produces species-typical defensive and escape behaviors (Neurosci. Abst., 1987, #127.1), and the present study examined whether a conditioned place aversion would develop in an environment in which the AHA -ESB was applied.

The experimental group (n=6 rats) received the AHA -ESB on their prefered side of the shuttlebox and no ESB on the other for four pairings. The control group (n=6) experienced the same procedures but with no ESB. The animals' side preference was then reassessed.

After 4 pairings of the ESB with the previously preferred side of the test chamber, a reliable decrease in side preference was exhibited on the first test after training [$F(1,10)=96.2$, $p<.001$], as well as 3 days [$F(1,10)=234.9$, $p<.001$] and 7 days [$F(1,10)=83.7$, $p=.001$] afterwards.

This research demonstrates the ESB of the AHA generates an aversive affective state that produces strong avoidance of an environment in which such stimulation was received. These results support the contention that ESB of AHA sites which yield the unconditioned response of flight does generate a true emotional state resembling fear. Therefore, the AHA may be part of a neural circuit which mediates both the behavioral manifestations and internal experience of this emotion.

444.4

IS ESCAPE FROM "REWARDING" BRAIN STIMULATION DUE TO NOCICEPTIVE PROPERTIES OF THE STIMULATION? J. Pollock, J.E.G. Williams and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston Univ. Sch. Med., Boston, MA 02118.

Prolonged electrical stimulation to rewarding brain sites will elicit escape behavior in rats. The present experiment was designed to determine if this escape behavior is reinforced by the termination of a nociceptive stimulus or reinforced by the rewarding effects of the onset of the next stimulus. If an analgesic drug having no effect on rewarding brain stimulation raises the threshold and an hyperalgesic drug, also having no effect on rewarding brain stimulation, lowers the threshold for escape from stimulation to rewarding brain areas, it would argue that such stimulation is nociceptive. In the present experiment we determined the effects of two drugs that met the above criteria, ethylketocyclazocine (EKC) and naloxone (NX) respectively, on the threshold for escape from stimulation to the medial forebrain bundle-lateral hypothalamic (MFB-LH) area. Results indicate that EKC (0.5-1.0 mg/kg) raises the escape threshold whereas NX (8.0-16.0 mg/kg) lowers the escape threshold, suggesting that aversiveness of electrical brain stimulation to the MFB-LH is the result of the nociceptive quality of stimulation and not the result of the rewarding effects of the onset of stimulation. [(Supported in part by NIDA grant DA 02326 and Research Scientist Award DA 00099 (CK) and a Culpepper Award (JP)].

444.5

SELECTIVE LESIONS OF THE DUAL OLFACTORY SYSTEM AND CAT SMELL-ATTENUATED PLAY BEHAVIOR AMONG JUVENILE RATS. L. Crepeau* & J. Panksepp (SPONSOR: J. ROSSI III). Department of Psychology, Bowling Green State University, Bowling Green, OH 43403

Juvenile rat play was measured in the presence and in the absence of cat smell in groups of rats following damage to the main, accessory or both olfactory systems, following intranasal infusions of zinc sulfate [ZINC], transections of the vomeronasal nerve [VNX], or olfactory bulbectomy [OBX], respectively. Control group animals [CON] received either sham surgery or intranasal infusions of distilled water.

During days 1-5 of testing, half of each group (CON, ZINC, VNX & OBX) were tested in clean wood chip bedding, while the other half of each group (CON_2, $ZINC_2$, VNX_2 & OBX_2) was tested in bedding containing cat odors, & conditions were reversed during days 6-10. Olfactory discrimination was also tested daily following 7-8 hours of food deprivation. Animals were allowed 3 min to find a bait buried in wood chip bedding inside a 22 X 36 X 25 cm aquarium. Both the control and VNX groups had intact smell sense, while the ZINC and OBX groups were severely impaired.

Compared to testing in clean bedding, cat smell reduced pinning levels in the CON and ZINC groups by 81 and 30 percent, respectively, and rough-and-tumble activity by 64 and 15 percent. Play levels in the VNX and OBX were unaffected by the presence of cat odors. Cat odors also suppressed pinning and activity levels in the CON_2 and $ZINC_2$ groups, an effect which persisted in clean bedding during test days 6-10, apparently from the initial cat smell experience. Rates of play in the VNX_2 and OBX_2 groups remained stable.

Disinhibition of play among VNX group animals in the presence of cat smell indicates that the AOS may elaborate behavioural inhibition in the presence of olfactory cues which indicate danger to the animal.

444.6

THE PARAMETRIC STUDY OF A TASTE AVERSION MODEL OF DRUG DISCRIMINATION (DD) LEARNING. T.V. Jaeger* and R.F. Mucha, Addiction Research Foundation, Toronto, M5S 2S1; and Dept. of Pharmacology, Univ. of Toronto, Canada.

At last year's meeting, data of Kautz etal. and Martin etal. suggested that taste aversion may provide a baseline for DD learning requiring high sensitivity, such as with naloxone in naive animals and with intracerebral drugs. Therefore, we manipulated and examined a model using SC drug to predict in thirsty rats (Sprague-Dawley males) whether or not an emetic follows presentation of 4 mls of palatable fluid. Differential consumption of the fluid was found during daily training with 0.04 mg/kg fentanyl or 20 mg/kg pentobarbital as the drug cue and 30 to 120 mg/kg LiCl as the emetic, although learning was slower using two trials per day. As DD was seen whether the drug predicted safety or discomfort, the results were not due to an acute interaction of the flavor and drug; however, learning was more rapid and in more animals with learned safety. Fentanyl dose generalization tests indicated that 0.005 mg/kg was distinguished from saline in many learned-safety rats, but 0.01 mg/kg or more was required in the learned discomfort subjects. Also, fentanyl-trained rats did not recognize pentobarbital as drug, or vice versa. Therefore, DD can, indeed, be studied with a taste aversion model, but the identification of parameters of high sensitivity may require more work.

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444.7

SOMATOSENSORY REGULATION OF NURSING BEHAVIOR IN RATS. J.M. Stern and S.K. Johnson*. Psychology Dept., Rutgers University, New Brunswick, NJ 08903.

Based on pup retrieval ability, maternal behavior in rats is thought to be under multisensory control, but the sensory regulation of nursing behavior has been neglected. We assessed the dam's display of the upright crouching posture, i.e., pronounced dorsal arch, splayed legs, and immobility, following manipulations meant to reduce perioral or ventral somatosensory stimulation from pups. Dams were separated from their litter for 4-6 h prior to the crouching test; pups were placed in the nest if retrieval ability was impaired.

Before nursing begins, dams typically engage in several oral behaviors, including retrieving, licking and rearranging pups. Deprivation of perioral stimulation from pups during early lactation (day 2) via local anesthesia of the dams' mystacial pads with lidocaine or via muzzling almost completely eliminated crouching (but not maternal interest), whereas all controls crouched, as did dams deprived of licking by mouth suturing. At 2 wks postpartum, when pups leave the nest and are active in initiating nursing bouts, perioral stimulation from the pups is not essential. At all stages of lactation, active nuzzling of the dam's ventrum by pups is required to elicit crouching. This was shown by providing dams with freshly killed, but still warm pups, with live but inactive, chilled pups, and with active pups rendered incapable of rooting effectively due to mystacial pad anesthesia. Such pups are retrieved, licked, and hovered over, but the crouching posture is not assumed. (Supported by NIMH Grant MH 40459.)

444.8

MEDIAL PREOPTIC AREA AFFERENTS AND MATERNAL BEHAVIOR IN THE RAT. M. Numan, J. McSparren*, and M.J. Numan*. Dept. of Psychology, Boston College, Chestnut Hill, MA 02167.

Knife cuts severing the lateral connections of the medial preoptic area (MPOA) disrupt maternal behavior in rats. In our first experiment we found that knife cuts which severed the dorsolateral connections of the MPOA disrupted maternal behavior as effectively as did full MPOA lateral cuts, while knife cuts which severed the ventrolateral MPOA connections were ineffective.

Our second experiment examined MPOA afferents severed by the lateral cuts. Full MPOA cuts, dorsolateral MPOA cuts, and ventrolateral MPOA cuts were made with a horseradish peroxidase (HRP) coated wire knife. Nuclear groups containing HRP labeled neurons after full and dorsolateral cuts, but not after ventrolateral cuts, will suggest areas afferent to the MPOA which may be important for maternal behavior. The results obtained so far indicate the presence of HRP labeled neurons in several regions. A preliminary finding of importance is that full and dorsolateral cuts label more cells in the posteromedial nucleus of the solitary tract (NST) than do ventrolateral cuts.

The results suggest that NST input to the MPOA may influence maternal behavior.

444.9

EFFECTS OF KAINIC ACID ON EMOTIONAL AND SENSORIMOTOR BEHAVIOR IN DOMESTIC CHICKS. L. Normansell, D. Zeisloft*, and J. Panksepp. Muskingum College, New Concord, OH 43762 and Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403.

Intraventricular administration of kainic acid (KA), a conformationally-restricted analog of the excitatory amino acid glutamate, has been shown to induce vocalizations in chicks (Soc Neurosci Abstr 13: 763, 1987). This capacity of KA to increase calling is more apparent when animals are tested under environmental conditions where baseline calling rates are generally reduced.

When chicks were tested alone surrounded by mirrors, in the dark in groups of four, when exposed to loud ambient sound levels, or when held in the cupped hands of the experimenter, KA-treated animals (.1-.25 µg) vocalized more frequently than did water-treated controls, suggesting that KA may disrupt processing of the visual, auditory, and somatosensory stimuli which influences call production.

Following KA administration, other behaviors of young chicks are also affected. KA-treated chicks fail to respond to a novel object placed into their immediate environment, they exhibit abnormal approach tendencies toward the remainder of their flock, they assume a fear-like squatting posture, and they make more errors than controls on a pebble floor task of visual discrimination.

Effects of KA on the current thresholds required to produce vocalizations by electrical stimulation was also investigated after stereotaxically-guided microinjection into the Midbrain Calling Region. Two min. after KA infusion (.5 µg), stimulation thresholds were increased about 18%, whereas thresholds in control animals tended to decrease. Following surgery, KA-treated chicks showed only a slight reduction in calling frequency when they were tested in isolation. Those animals, however, did not respond to the presence of their own mirror images by a suppression of calling, suggesting a long-term disruption of visual capability.

444.10

ETHOLOGICAL STUDY OF OPEN-FIELD BEHAVIOR. P. Kabai*, A. Lajtha, G. Kobor* and C. Vadasz. Neurochem. Div., The Nathan S. Kline Inst., Orangeburg, NY 10962.

Behaviors which follow each other in the behavior sequence by higher probability than chance tend to correlate positively, thus masking underlying motivational factors. A statistical method was developed to correct for the sequential effects on correlations among behaviors in the open-field (OF) test.

OF behavior of 200 mice, a complete generation of a longterm neurogenetic study, starting from F_2 generations of 3 inbred strains (Vadasz, C. et al, J. Neurogen., 4:241-252, 1978), was monitored using an event registration software, ETHOGRAM, developed in our laboratory. Occurence of 16 behaviors was recorded. Behavior sequences were higher than second order Markov chains i.e. the probability of the occurence of a behavior is dependent at least on the two preceding acts. Factor analysis of the corrected correlation matrix yielded 6 factors which were labeled as EXPLORATION (rearing and leaning), ESCAPE (climb and jump on wall), DIRECTION CHANGE (backstep while rearing and turn), URINATION and two GROOMING factors. Defecation had a negative loading on one of the grooming factors and a small positive one on "escape".

These results demonstrate that the narrow interpretation of OF tests (in terms of EXPLORATION and EMOTIONALITY) is an oversimplification of the complexity of OF behavior.

444.11

SELECTED LINES OF RATS DIFFER IN THEIR RESPONSIVITY TO SUCCESSIVE NEGATIVE CONTRAST AND BENZODIAZEPINE TREATMENT. G.A. ROWAN & C.F. FLAHERTY. Psychology Dept, Rutgers Univ. New Brunswick, N.J. 08904

The consummatory behavior of rats shifted from 32% to 4% sucrose declines to a level substantially below that of unshifted rats that have experienced only 4% sucrose. This negative contrast effect involves a stress component (corticosterone elevation) and the decrement is attenuated by administration of the benzodiazepine tranquilizers.

The present experiments investigated the behavior of two selected lines of rats that have been shown to differ in "emotionality". The Maudsley Reactive (MR/Har) and Maudsley Nonreactive (MNRA/Har) rats and the Syracuse High and Low Avoidance rats (SHA/Bru and SLA/Bru) were both compared to a large population (N=397) of nonselected rats in terms of their negative contrast effect. The results demonstrated that the "nonemotional" rats in both selected lines (SHA and MNRA) showed reliably smaller decrements in licking when shifted from 32% to 4% sucrose than is typical in unselected rats. The SLA rats (high emotional) showed marginally larger contrast effects than the unselected rats. However, the MR rats, selected for open field defecation, showed significantly smaller contrast effects than the unselected population.

Chlordiazepoxide (8 mg/kg) was differentially effective in reducing the negative contrast in the selected lines.

444.12

EFFECTS OF ACUTE PHENYTOIN ON CORTICAL EVENT-RELATED POTENTIALS. E.S. Barratt, S.A. Shappell, M.E. Brandt*. Dept. of Psychiatry and Behavioral Sciences, University of Texas Medical Branch, Galveston, Texas 77550-2777.

Acute dosages of phenytoin have been shown to have an effect on both the early and late cortical event-related potentials while subjects observed light flashes at three intensities (Barratt, et al., Neuropsychobiology, 15:201-207, 1986). In this double-blind, placebo controlled study, phenytoin (100mg) significantly decreased the amplitude of the N100 component (reducing) and enhanced the frontal negative portion of the slow wave but did not effect the posterior portion of the P300 wave form. The current study extended this research by: (1) using 2 dosages of phenytoin (100mg and 200mg); (2) having subjects perform 2 additional tasks (oddball and go/no-go). The phenytoin related regional differences in ERP's were consistent with the previous study at both dosages. However, the 200 mg dosage had more generalized cortical effects. These experiments involved the use of a topographical analysis of EEG's (Cappola, et al., Comput Biol Med, 12:191-199, 1982). The results are consistent with the hypothesis that phenytoin effects cognition by acting on frontal cortical areas during information processing.

444.13

UNIT ACTIVITY IN THE HIPPOCAMPUS AND TEMPORO-BASAL CORTEX RELATED TO ATTENTION AND MEMORY IN THE BEHAVING MONKEY. T.R.Vidyasagar*,E.Salzmann* and O.D.Creutzfeldt(SPON:J.Kulikowski).Max Planck Institute for biophys. Chem.,Göttingen, FRG.

Single and multiunit activities were recorded from the hippocampus and temporo-basal cortex, as the monkey(Macaca fascicularis) was performing a delayed match-to-sample (DMS) task or during a variety of behaviour situations that involved attention, expectation, reward or position in egocentric space. Only a minority of neurones gave significant responses related to the DMS task. In contrast, many cells responded vigorously to situations involving the experimenter or particular emotional or motivational states of the animal. For example, many units responded (or were inhibited) during the time the monkey was shown a piece of food that was about to be given to him or while consuming it. Others were activated or inhibited when the experimenter entered or left the room, sometimes the response being dependent on the location of the event relative to the monkey. It is possible to interpret the responses in the DMS tasks as being more related to changes in motivational or attentional states of the animal during the task than to the process of memory storage itself.

444.14

THE EFFECTS OF BENZODIAZEPINES, 5HT1A AGONISTS AND ETHANOL ON NATURAL ANXIETY/DEFENSIVE BEHAVIORS OF RATS TO A CAT. R.J. Blanchard, D.C. Blanchard* and J. Rodgers. Psychology Department, Univ. of Hawaii, Honolulu, HI 96822.

Rats living in a Visible Burrow System connected to a "surface" area show a complex pattern of changes when a cat is briefly placed in the surface area, or when a cloth containing cat odor is left there. Initial patterns of withdrawal and avoidance include flight from this area to the tunnels, freezing in the tunnel/burrow system, and avoidance of openings to the surface area. These give way to a pattern of risk assessment. Nondefensive behaviors including grooming, eating, drinking and aggression are inhibited.

We are examining the effects of GABA/BZP compounds, 5HT1A agonists, and ethanol on the various behaviors measured in this situation. Results indicate that these three anxiolytic compounds produce different profiles on this task.

444.15

INFLUENCE OF TASK-COMPLEXITY AND SUBJECTIVE ESTIMATES OF INTEREST VALUE ON EVENT-RELATED POTENTIALS TO COMPLEX VISUAL STIMULI

V. Hömberg * and H. Schuhmacher*, University of Düsseldorf (SPON: European Neuroscience Association).

We have shown before that several late positive components of event-related potentials (ERP) to complex visual stimuli are systematically related to subjective experience of interest value. (Hömberg et al., Ann. NY. Acad. Sci. 425: 216-222).

This study was designed to look at the influence of difficulty of semantic processing, subjective scalings of interest value and stimulus repetition on ERPs to complex visual stimuli. 7 male volunteers were confronted with up to 500 objects presented tachistocopically for 150 ms. EEG was taken from various mid-line electrodes referred to linked mastoids. Averages were composed over tasks of various complexity and according to subjective estimates of interest value.

Principle component analyses revealed multiple partially overlapping late positive components: a parietally prepondenant P300 and a fronto-centrally dominant P800 were systematically related to demand of semantic processing as well as to psycho-physical estimates of interest value. Later prefrontally dominant positive component (LPC) was related to the difficulty of processing but not to interest value. P300, P800 and LPC simularly habituated with repetitive presentation of the same stimulus.

Multiple long latency ERP with different topography are systematically related to difficulty of semantic processing.and subjective experience of interest value.

FEEDING AND DRINKING VII

445.1

OPIOID INJECTIONS INTO THE BED NUCLEUS OF THE STRIA TERMINALIS: EFFECTS ON FOOD INTAKE.

B.A. Gosnell. Dept. of Psychiatry, University of Michigan, Ann Arbor, MI 48109-0116.

The stria terminalis is a major efferent pathway from the amygdala, and enkephalin-containing fibers have been demonstrated in this pathway and in the bed nucleus of the stria terminalis (BNST) (e.g., Uhl et al., Brain Res. 149:223-228, 1978). In this report, the possibility was tested that the BNST may contribute to the increased feeding observed after systemic or icv injections of opioid agonists. Cannulas were implanted unilaterally into the BNST of male Sprague-Dawley rats. In a repeated measures design, the mu opioid agonist DAGO was injected into the BNST at doses of 0 (NaCl), 0.1, 0.3, and 1 nmol. In a separate group of rats, the selective delta agonist DTLET was tested at the same doses. The 1 nmol dose of DAGO caused a small but significant increase in 2 and 4 hr food intake. Intake was also increased after DTLET (1 nmol), although the effect fell just short of statistical significance. These experiments suggest participation of the BNST in the control of food intake. However, due to the proximity of the BNST to the lateral ventricle, it is possible that the effects are due to a diffusion of the peptides into the ventricle and an action at other brain sites. Studies are in progress to determine whether the BNST and stria terminalis play a role in the stimulation of intake by injections of DAGO into the amygdala (Gosnell, Neuropharmacol. 27:319-326,1988).
(Supported by NIH Grant NS23565)

445.2

NEW PHENYL PIPERIDINE OPIOID ANTAGONISTS: THEIR EFFECT ON MU AND KAPPA ANALGESIA IN MICE AND ON FOOD CONSUMPTION IN THE OBESE ZUCKER RAT.

C.H. Mitch*, D.M. Zimmerman*, L.G. Mendelsohn, W. Shaw*, B.E. Cantrell*, J. Reel*, J. Snoddy* and J.D. Leander. Lilly Research Laboratories, Indianapolis, Indiana 46285. (SPON: M.J. Schmidt)

Following our initial discovery of potent pure opioid antagonist activity within the trans-3,4-dimethyl-4-phenylpiperidine series (D.M. Zimmerman, et al, Nature, 275, 332, 1978), we have greatly expanded the SAR in an effort to find new opioid antagonists with enhanced selectivity. A variety of trans-3,4-dimethyl-4-phenylpiperidines differing in the nitrogen substituent were synthesized. These were evaluated as opioid antagonists and for their effect on food consumption in the fatty Zucker rat. While several of these compounds were found to have exceptional potency as opioid antagonists and in reducing food consumption, LY255582 [(3S,4R)-1-((S)3-hydroxy-3-cyclohexylpropyl)-4-(3-hydroxy-phenyl)-3,4-dimethyl-1-piperidine) emerged as having the best overall activity profile. It has an affinity for mu and kappa receptors of 0.41 nM and 2.02 nM, respectively. In mice, it antagonizes 1.25 mg/kg (s.c.) morphine (mu) and 2.5 mg/kg (s.c.) U-50, 488 (kappa) induced analgesia with AD50 values of 0.01 mg/kg (s.c.) and 0.07 mg/kg (s.c.), respectively. In the Zucker rat, a dose of 0.05 mg/kg (s.c.) reduced 4 hour food consumption to 80% of control. LY255582 has promise as a potent opioid antagonist and appetite suppressant.

445.3

EFFECTS OF OPIATES AND THEIR ANTAGONISTS ON WATER INTAKE OF POLYDIPSIC INBRED MICE.

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Previously we have reported that the sensitivity of neurons in the subfornical organ (SFO) and AV3V of the polydipsic strain of mice (STR/N) differs from that of controls (non-polydipsic, STR/IN and Swiss/Webster mice). The primary polydipsia, which amounted to 3 to 5 times that of controls, was greatly reduced by administration of naltrexone in polydipsic strain but not in controls. Following intracerebroventricular injection of naltrexone through minipump for 7 consecutive days, the earlier reduction in drinking was followed by a rebound in which drinking nearly doubled the amount observed in pre-injection period. This continued for more than 6 weeks after the end of the injection in this special strain. Recordings from neurons in the AV3V and the SFO in brain slice preparations showed that in all 3 groups of mice the opiates inhibited the neuronal activity, but the threshold for inhibitory action was higher in polydipsic than that found in the other two groups of mice, STR/IN and Swiss/Webster. Naltrexone or naloxone applied to the bathing medium did not change the activity of these neurons. Our results suggest the involvement of both angiotensin II and opiates in the polydipsia observed in this special strain (Supported by NIH,USPHS NS- 00847).

445.4

INTERACTION OF THE ENDOGENOUS OPIOID SYSTEM AND RADIATION IN THE SUPPRESSION OF APPETITIVE BEHAVIOR,

D.E. Morse, and G.A. Mickley, (SPON: J. McDonough) Dept. of Behavioral Sciences, AFRRI, Bethesda, MD 20814-5145

Endogenous opioids modify a wide variety of behaviors, including 'appetite'. Radiation exposure and other stressors have been reported to induce the release of endogenous opioids. Suppression of appetitive behaviors and loss of body weight are frequent consequences of radiotherapy (as used in cancer treatment). Still, the literature contains no information on the role of the endogenous opioids in postirradiation 'appetite' suppression. The present study examined interactions between the mu opioid system and radiation in post-exposure suppression of appetitive behaviors. Chronic opioid system blockade, with naltrexone, was found to partially ameliorate suppression of appetitive behaviors during the first 24 hours following radiation exposure. Post-exposure treatment with naltrexone was found to have nonsignificant effects on behavioral suppression. Administration of morphine was also found to have limited impact on the dose-effect and time-course of post-irradiation suppression of appetitive behaviors. The data suggest that changes in mu opioid system activity probably do not contribute to radiation induced 'appetite' suppression. Furthermore, the data suggest that, in clinical settings, pain control medications may not exacerbate radiotherapy induced appetite suppression.

445.5

ANTIBODIES TO DYNORPHIN A [1-8] AND [1-17] ELEVATE THRESHOLD FOR BRAIN STIMULATION-INDUCED FEEDING IN RAT. K.D. Carr, T.H. Bak* and E.J. Simon (SPON: M. Holland). Dept. of Psychiatry, NYU Med. Ctr., NY NY 10016.

We have previously reported that antibodies to DYN A [1-13] infused into the lateral ventricle (LV) produce a naloxone-like elevation in threshold for electrical brain stimulation-induced feeding (SIF) (Carr et al., Brain Res 422: 384, 1987). In the present study, antibodies to DYN A [1-8] and [1-17] were infused into the LV or meso-pontine aqueduct (AQ) to investigate the CNS level and DYN fragment that mediate SIF. LV infusions of antibodies to DYN [1-17] elevated the SIF threshold ($\bar{X}$=+23.6%; SEM=5.7; N=10) as did antibodies to DYN [1-8] ($\bar{X}$=+17.5%; SEM=7.3) when compared with the effect of normal rabbit serum ($\bar{X}$=-5.4%; SEM=3.5; N=5). AQ infusion of antibodies to DYN [1-8] produced a pronounced elevation in threshold ($\bar{X}$=+30.9%; SEM=5.9; N=5) as compared with normal serum ($\bar{X}$=+7.5%; SEM=10.6; N=5), while antibodies to DYN [1-17] did not ($\bar{X}$=+12.9%; SEM=9.7).

In our SIF paradigm, naloxone produces a marked pattern of progressive elevation in serially determined thresholds (Carr & Simon, Brain Res. 297:369, 1984). In the present study, a similarly marked pattern was produced only by DYN [1-8] antibodies infused into the AQ.

These results suggest that DYN [1-17] and [1-8] may both mediate SIF, and that DYN [1-8] activity in some caudal periventricular structure(s) may contribute prominently to the opioid mediation of feeding as revealed by naloxone antagonism. (Supported by NIDA grant DA03956 to K.D.C.)

445.6

OPPOSITE EFFECTS OF ROSTRAL AND CAUDAL VENTRICULAR INFUSION OF NOR-BINALTORPHIMINE ON STIMULATION-INDUCED FEEDING. T. Bak*, K.D. Carr, E.J. Simon and P.S. Portoghese#. Dept. of Psychiatry, NYU Med. Ctr., NY NY 10016 and #Dept. of Med. Chem., Univ. Minn. Med. School, Minneapolis, MN 55455.

The electrical brain stimulation threshold for eliciting feeding is elevated by centrally administered antibodies to the putative endogenous kappa ligand, dynorphin A (Carr et al., Brain Res. 422:384, 1987). In the present study, nor-binaltorphimine (nor-BNI), a selective kappa receptor antagonist (Portoghese et al., Life Sci. 40:1287, 1987), was used to evaluate kappa mediation of stimulation-induced feeding in the rat.

Lateral ventricular (LV) infusion of nor-BNI (50 ug) elevated mean stimulation frequency threshold from 16.5 pps (± 1.2; N=7) to 21.6 pps (± 2.6). A 25 ug dose of nor-BNI had no effect. Naloxone (100 ug) elevated threshold from 15.5 pps (± 1.2; N=6) to 18.1 pps (± 1.0).

When antagonists were delivered via the meso-pontine aqueduct (AQ), nor-BNI (50 ug) reduced the feeding threshold from 16.5 pps (± 1.4; N=4) to 13.2 pps (± 1.0), while naloxone still elevated threshold. AQ infusion of the selective kappa agonist, U50,488 (100 ug & 200 ug) produced a modest elevation in threshold [e.g. from 17.9 pps (± 1.8) to 20.4 pps (± 2.4)].

These results suggest that kappa opioid activity in rostral periventricular structures may facilitate feeding while kappa activity in caudal periventricular structures may inhibit feeding. (Supported by NIDA grant DA03956 to K.D.C.)

445.7

EFFECTS OF EXERCISE & RESTRICTED FEEDING ON WEIGHT LOSS AND BETA-ENDORPHIN LEVELS IN THE RAT: IMPLICATIONS FOR ANOREXIA NERVOSA. L.E. Doerries*, P.F. Aravich, A. Metcalf*, J.D. Wall* and T.J. Lauterio* (SPON: D.B. West). Dept. Psychology, Christopher Newport College, Newport News, VA 23606; Dept. Anatomy & Cell Biology and Dept. Internal Medicine, Eastern Virginia Medical School, Norfolk, VA 23510.

It has been proposed that exercise may be a risk factor for anorexia nervosa. It also has been proposed that anorexia nervosa is related to elevated beta-endorphin levels. We have begun a systematic examination of the phenomenon of activity-based anorexia in which exercise produces profound, voluntary anorexia in rats maintained on restricted-feeding schedules. The phenomenon also has been related to increased beta-endorphin levels. We now report that: 1) activity-based anorexia is associated with reliable elevations in circulating beta-endorphin compared to restricted feeding without exercise; 2) absolute arcuate hypothalamic beta-endorphin, although tending to be lower, is not affected reliably (beta-endorphin content relative to tissue protein will be reported later); and 3) chronic naloxone infusion (SQ) does not prevent the development of activity-based anorexia and, in fact, potentiates it. We conclude that beta-endorphin alterations are correlated with activity-based anorexia, but do not precipitate the syndrome.

445.8

EFFECTS OF EXERCISE & RESTRICTED FEEDING ON GLUCOPRIVIC EATING IN THE RAT: IMPLICATIONS FOR ANOREXIA NERVOSA. P.F. Aravich, L.E. Doerries*, E. Stanley*, A. Metcalf* & T.J. Lauterio*. Dept. Anat. & Cell Biol. and Dept. Int. Med., Eastern Virginia Med. Sch., Norfolk, VA 23510; Dept. Psychol., Christopher Newport College, Newport News, VA 23606.

Anorexia nervosa (AN) is associated with a variety of neuroendocrine and behavioral disorders, including an impaired ingestive response to 2-deoxy-D-glucose (2DG) glucoprivation. Unfortunately, it is often difficult to distinguish between the primary abnormalities of AN versus the secondary consequence of severe emaciation. Because of increased interest in the relationship between exercise and AN, we are beginning a systematic examination of "activity-based anorexia" in the rat, which is produced by superimposing exercise on a restricted-feeding schedule. We now report that activity-based anorexic rats differ from body-weight matched control rats with respect to the behavioral response to a 2DG challenge. Specifically, whereas 2DG-treated activity-based "anorexic" rats fail to increase food intake relative to saline-treated "anorexic" rats (9±1 vs. 10±2 g, respectively; p>.05), 2DG-treated body-weight matched animals reliably decrease food intake compared to saline-treated body-weight matched controls (8±1 vs. 12±1 g, respectively; p<.05). Beta-endorphin responses as a function of treatment condition will be reported later.

445.9

NALOXONE POTENTIATION OF EFFECTS OF CHOLECYSTOKININ (CCK) AND LiCl ON OXYTOCIN (OT) SECRETION, GASTRIC MOTILITY AND FEEDING. L.M. Flanagan, J.G.Verbalis, E.M. Stricker. Depts. of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260

Neurohypophyseal secretion of OT in response to dehydration, hypovolemia, and parturition in rats is known to be potentiated by the opioid antagonist naloxone (NX). The present studies demonstrated that stimulation of OT secretion by systemic injections of CCK and LiCl likewise is potentiated by NX. Moreover, the inhibitory effects of CCK and LiCl on gastric motility and feeding similarly were enhanced by NX. Because neurohypophyseal hormone secretion and inhibition of gastric motility are known to be mediated by oxytocinergic neurons projecting from the paraventricular nucleus of the hypothalamus, this parallel potentiation by NX of CCK- and LiCl-induced effects on OT secretion, gastric motility, and food intake suggests that: 1) OT pathways are likely involved in some aspects of central control of feeding behavior, and 2) endogenous opioid peptides are likely involved in regulation of this and other oxytocinergic neuronal systems in rat brain.

Supported by research grant MH-25140, and training grant MH-18273-03.

445.10

CHOLECYSTOKININ (CCK) SUPPRESSES GASTRIC MOTILITY BUT MAY NOT STIMULATE PITUITARY OXYTOCIN (OT) SECRETION IN LACTATING RATS. E. Thiels, D. L. Helmreich, J. G. Verbalis, E. M. Stricker. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, PA 15260.

CCK is known to cause a correlated inhibition of gastric motility and stimulation of OT secretion in male rats. We report here the effects of CCK on gastric motility and OT secretion in virgin females and lactating rats 8 to 16 days postpartum. CCK given ip decreased gastric motility in both groups of female rats: by 40-60% for 10-20 min after 0.01 ug/kg CCK and by more than 60% for longer than 60 min after 10 ug/kg CCK, values comparable to those seen in male rats. In contrast, 1-10 ug/kg CCK increased plasma OT levels by 3-10 uU/ml in virgin females as in male rats, but had no effect on OT secretion in lactating rats. These results suggest that hypothalamic magnocellular neurons in lactating rats may become insensitive to the effects of systemic CCK, whereas the hypothalamic parvocellular neurons that mediate inhibition of gastric motility remain fully responsive to CCK. It is not yet clear whether this apparent dissociation between pituitary OT secretion and inhibition of gastric motility also is seen when lactating rats are given other treatments that elicit both responses in male and virgin female rats. (Supported by NIMH research grant MH-25140.)

445.11

THE EFFECT OF CHOLECYSTOKININ OCTAPEPTIDE ON FOOD INTAKE AND MATERNAL BEHAVIOR OF LACTATING AND POSTWEANING RATS. S.A. Wager-Srdar and A.S. Levine. Neuroendocrine Research Laboratory, VA Medical Center and University of Minnesota, Minneapolis, MN, 55417.

Cholecystokinin octapeptide (CCK-8) decreases food intake in a number of species. Behavioral studies have shown that CCK-8 elicits a sequence of behaviors similar to those of post-prandial satiety. It has been found that CCK-8's satiating effect is altered during lactation. We examined the effect of CCK-8 (5 ug/kg) (ip) on early dark cycle food intake and maternal behaviors of rats in early, mid and late lactation and the postweaning (PW) period. Food intake was decreased by CCK-8 during early [$F(1,99) = 4.017$] and during mid [$F(1,99) = 6.80$], but not late lactation or PW and these findings are similar to those of earlier studies. During lactation and the PW period, the rate of food consumption was decreased by CCK-8 compared to the saline group [$F(1,30) = 19.59$]. The average meal length was not altered by CCK-8. However, the latency to the first meal was increased 33-47% during lactation but not during PW. The maternal behaviors, nursing and pup interaction were not altered by CCK-8. Resting and sleeping behaviors were increased by CCK-8 [$F(1,30) = 24.04$], and exploratory behavior was decreased [$F(1,30) = 17.59$]. CCK-8 altered food intake during lactation by decreasing the rate of consumption and increasing the latency time to the first meal. Maternal behavior was not affected by CCK-8 in this study.

445.12

INSENSITIVITY OF DIABETIC (DB) RATS TO CHOLECYSTOKININ (CCK)-INDUCED SATIETY. M.A. DellaFera and B.D. Coleman*. Dept. Int.Med. Washington U. Sch.Med. St. Louis, MO 63110

Streptozotocin-induced diabetes in rats results in development of hyperphagia and changes in exocrine pancreatic and gastrointestinal function. The following study was carried out to determine the relative sensitivity of DB and control (C) rats to satiety induced by CCK-8 administered IP, and to determine changes in sensitivity over time after induction of diabetes. Male Sprague Dawley rats (6/group) were given 0, 2 and 4 ug/kg CCK-8 IP (Latin square design) after a 6 hr fast on weeks 1, 3, 5, 7, and 9 after administration of streptozotocin (S) or control (C). As early as 1 wk after S, DB rats failed to respond to CCK-8, whereas there was a clear dose-response effect in C rats. By week 9 there was still no significant decrease in food intake for DB rats, although there was trend toward a decrease; C rats still exhibited a significant dose-related decrease in intake. Diabetes results in increased rate of gastric emptying, a function partially controlled by CCK induced contraction of the pyloric sphyncter. The slowing of emptying results in distention, which may be an important signal of satiety. Defects in CCK receptor mechanisms have been identified in the pancreas of DB rats; if the same is true for pyloric CCK receptors, this could be a possible mechanism for the insensitivity to CCK's satiety effect. Supported by NIH NS20000 and Monsanto Co.

445.13

CHOLECYSTOKININ (CCK) ANALOGS AS PROBES IN ASSESSING THE SITE OF ACTION OF CCK SATIETY. T.H. Moran, T.K. Sawyer*, R.T. Jensen*, R.J. Crosby* and P.R. McHugh. Department of Psychiatry, Johns Hopkins Univ, Baltimore, MD, 21205. Biopolymer Chemistry, Upjohn Co., Kalamazoo, MI, 49001. Digestive Diseases Branch, NIH, Bethesda, MD, 20892.

Roles for both pyloric and vagal CCK receptors in the mediation of CCK satiety have been proposed. The present work examines two CCK analogs for differences in their affinity for pyloric and vagal CCK receptors, potency in stimulating in vitro pyloric contraction and in inhibiting feeding as a means to identify the site of action of CCK satiety. The analog structures are: 1) Ac-Asp-Tyr[SO_3H]-Nle-Gly-Trp-Nle-Asp-Phe-NH_2, 2) Ac-Asp-Phe[p-NH_2]-Nle-Gly-Trp-Nle-Asp-Phe-NH_2

	IC_{50} pyloric binding	IC_{50} vagal binding	EC_{50} pyloric contraction	Threshold 30 min feeding	Threshold 60 min feeding
CCK	1.1 nM	0.7 nM	0.8 nM	0.5 ug/kg	5.0 ug/kg
1	0.4 nM	0.5 nM	0.7 nM	.01 ug/kg	.01 ug/kg
2	720 nM	29 nM	>500 nM	>100 ug/kg	--

The Nle substitution does not alter the affinity for the receptor populations, but does significantly increase the satiety potency. However, the Phe[p-NH_2] substitution results in differential affinity for the receptor populations. Its lack of potency in in vitro and feeding tests is consistent with a pyloric site for CCK satiety.

445.14

HIGH DORSAL COLUMN TRANSECTION ATTENUATES BOMBESIN-INDUCED SUPPRESSION OF FOOD INTAKE. Ellen E. Ladenheim and Robert C. Ritter. Dept. of VCAPP, Washington State University, Pullman, WA 99164

The suppression of food intake after peripheral administration of bombesin (BBS) is partially mediated by spinal-visceral afferent neurons (Stuckey et al., 1985). However, sensory information from abdominal structures can utilize several different spinal pathways. In order to ascertain the importance of the dorsal column-medial lemniscal system in BBS-induced suppression of feeding we examined the ability of BBS to suppress short-term food intake in rats with high dorsal column transection. We have found that the suppression of feeding induced by peripherally administered BBS was significantly attenuated in rats with dorsal column transection compared to sham-operated controls ($p < .02$). Intraperitoneal injection of 4ug/kg BBS reduced 30 min intake of a palatable solid food (cookies) by 63.1% $\pm$ 5.8 in control rats while those with dorsal column transection reduced intake by 41.6% $\pm$ 7.5. Food intake following a baseline injection of 0.9% saline was not significantly different between groups ($p > .05$) These results indicate that high spinal cord transection of the dorsal column pathway interrupts a population of neurons which participate in BBS-induced suppression of feeding.

DRUGS OF ABUSE V

446.1

BUPRENORPHINE DETOXIFICATION IN OPIOID ADDICTS: DOSE EFFECTS. T.A. Kosten, J.H. Krystal, C.M. Morgan*, H.D. Kleber*, and T.R. Kosten*. Dept. of Psychiatry, Yale Univ., New Haven, CT 06519.

Pharmacological treatment of opioid addicts have used either methadone (MET), an opiate agonist, or naltrexone (NLX), an opiate antagonist. Successful detoxification from MET to NAL has been limited. Buprenorphine (BUP) is a partial opioid agonist which at higher doses can act as an opioid antagonist. An optimal treatment dose would substitute for MET without withdrawal symptoms, block opioid induced euphoria, and then allow detoxification to NLX. To determine this dose of BUP, we assigned 39 opiate addicted patients to one of three dose groups: 2, 4 or 8 mg. BUP dose was not related to treatment retention or to frequency of illicit opioid use: less than 20% of the patients left and only 22% of the urines contained opioids. Patterns of withdrawal symptoms differed across groups with the 4 mg group showing a decline in symptoms as shown by the significant effects of time ($p<.01$) and dose x time ($p<.05$). A moderate dose of BUP shows promise as a transitional agent from MET maintenance to NAL abstinence without causing serious withdrawal symptoms.

446.2

L-HISTIDINE BLOCKS THE EFFECTS OF PENTAZOCINE ON BRAIN-STIMULATION REWARD. S. Rassnick*, C. Decker* and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Previous results indicate that the antihistamine tripelennamine potentiates the threshold lowering effects of pentazocine (PEN) (Pharm. Biochem. Behav. 21:961, 1984) on brain-stimulation reward, a model of drug-induced euphoria. To determine histamine's role in this interaction we studied the effects of the combination of L-histidine (LHIS) and PEN on the threshold for brain-stimulation reward. As in previous studies PEN alone (2.5-10.0 mg/kg) lowered the threshold for rewarding stimulation to the medial forebrain bundle-lateral hypothalamus in male F-344 rats. Also LHIS (500 and 750 mg/kg) by itself had no significant effects, yet antagonized the threshold lowering effects of PEN. This interaction may be selective for opioids with kappa receptor activity since LHIS failed to antagonize the threshold lowering effects of morphine (Fed. Proc 46(3):404, 1987). These results suggest that central histamine may be involved in the reinforcing effects of PEN. [(Supported in part by NIDA grant DA 02326 and Research Scientist Award DA 00099 (CK)].

446.3

PRECIPITATED WITHDRAWAL IN MONKEYS AFTER REPEATED DAILY ADMINISTRATION OF DIFFERENT BENZODIAZEPINES.

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Relative physical dependence potential of four marketed benzodiazepines was assessed in monkeys in a flumazenil-precipitated withdrawal paradigm after the repeated oral administration of doses previously shown to be equieffective in producing a loss of righting reflex.

Squirrel monkeys received 18 daily oral administrations of 2 mg/kg alprazolam (A; N=4), 30 mg/kg diazepam (D; N=4), 1 mg/kg flunitrazepam (F; N=4), 280 mg/kg oxazepam (O; N=5) or vehicle (N=4). IV flumazenil injection 5 hours after the ninth daily treatment precipitated withdrawal (convulsions, tremor and/or vomiting were observed during a subsequent 1 hr period) in 1 A-, 4 D-, 1 F-, 4 O-, and no vehicle-treated monkeys. A second IV flumazenil injection given 5 hours after the eighteenth daily treatment resulted in withdrawal in 4 A-, 4 D-, 3 F-, 3 O-, and no vehicle-treated monkeys.

Thus, repeated oral administration of A, D, F, or O at equieffective doses produced physical dependence which was manifested as flumazenil-induced withdrawal signs in the majority of monkeys.

446.4

EFFECTS OF OPIATE AND DOPAMINERGIC DRUGS ON NOVELTY PREFERENCE BEHAVIOR.

M. T. Bardo, J. L. Neisewander and R. C. Pierce*. Dept. of Psychology, University of Kentucky, Lexington Kentucky, 40506.

Previous research has demonstrated that animals prefer a novel environment over a familiar environment. The present studies investigated the role of opioid and dopamine systems in novelty preference behavior. Rats were familiarized with a distinct environment by being confined to that environment for 30 min per day on eight consecutive days. On the ninth day, rats received either morphine sulfate (0, 0.1, 0.3, 1.0 or 3 mg/kg, s.c.), naltrexone hydrochloride (0, 0.1, 0.3 or 1.0 mg/kg, s.c.), amphetamine sulfate (0, 0.1, 0.3 or 1.0 mg/kg, s.c.) or haloperidol (0, 0.03, 0.1, 0.3 or 1.0 mg/kg, i.p.). Thirty min after injection, rats were allowed free-choice access to the familiar environment and to a novel environment for 15 min.

As expected, saline-injected control animals spent significantly more time in the novel environment than in the familiar environment. More important, the novelty preference behavior was counteracted in a dose-dependent manner by haloperidol, whereas amphetamine, morphine and naltrexone had no significant effect. These results indicate that novelty preference behavior involves a dopaminergic substrate.

(Supported by USPHS grant DA 05312.)

446.5

STIMULUS PROFILES OF AGONIST-ANTAGONIST OPIOIDS IN A DISCRIMINATION AMONG TWO DOSES OF MORPHINE AND SALINE IN PIGEONS.

A.M. Young, M.A. Walton, and A.N. Perkins. Dept. of Psychology, Wayne State University, Detroit, MI 48202.

Experiments evaluated the discriminative stimulus profiles of selected opioids in pigeons trained to discriminate among saline and two doses of morphine (MS). Pigeons (N=15) were trained to discriminate among i.m. injections of saline (SAL), 1.8 mg/kg MS (the low dose or LD cue), and 10 mg/kg MS (the high dose or HD cue). Performance was maintained under FR 30 schedules of food delivery in 30 min sessions. After establishment of stimulus control, various doses of MS, alone and in combination with naltrexone, and doses of other opioids were tested for generalization. For MS itself, LD generalization predominated at doses of 1.0 to 3.2 mg/kg; HD generalization at doses of 5.6 to 32 mg/kg. Naltrexone pA2 values for antagonism of the LD and HD cues were similar, suggesting that the discrimination was based on quantitative differences between the LD and HD cues. The agonists etorphine and methadone and the agonist-antagonists dezocine and GPA 1657 evoked dose-dependent generalization to both the LD and HD cues. In contrast, the agonist-antagonists nalbuphine and nalorphine evoked generalization to only the LD cue and, in other experiments antagonized the HD cue. These profiles suggest that a discrimination among saline and different MS doses may provide information about the agonist efficacy of agonist-antagonist opioids. (Supported by DA03796.)

446.6

ORAL SELF-ADMINISTRATION OF ETHANOL FACILITATES REWARDING ELECTRICAL BRAIN STIMULATION.

M. Moolten, G.T. Bain and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Many substances, including ethanol, which are readily abused in humans, have been reported to increase the sensitivity of animals to rewarding brain stimulation, a model of drug-induced euphoria. The reported effects of ethanol are less than robust, and notably variable. This variability may be due to differences in testing procedures as well as stress due to route of ethanol administration (typically intraperitoneal). In an attempt to resolve some of these ambiguities we employed a rate free threshold procedure with rats that orally self-administered ethanol.

Bipolar stainless steel electrodes were stereotaxically implanted in the medial forebrain bundle of male F-344 rats. Animals were water deprived, and then allowed to drink an ethanol-sucrose solution prior to testing. Significant threshold lowering effects were observed, which persisted throughout the testing session, at doses between 0.8 g/kg and 1.6 g/kg depending on the individual animal's sensitivity. These threshold lowering effects in animals self-administering ethanol suggest that the same pathways that subserve the reinforcing effects of other abused substances may be involved, at least in part, in the reinforcing effects of ethanol. [(Supported in part by NIAAA grant AA05950 and Research Scientist Award DA 00099 (CK)].

446.7

NALOXONE-INDUCED REDUCTION OF VOLITIONAL ETHANOL INTAKE CORRELATES WITH SPONTANEOUS PREFERENCE.

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Recently, the possibility of a common mechanism for the reinforcing properties of ethanol and opiates has been proposed on the basis of biochemical and behavioral data. This prompted us to study the effect of the administration of naloxone (NAL), an opiate antagonist, and Tyr-MIF-1, a brain peptide with anti-opiate properties, on ethanol intake in rats with a free choice of water.

In the first study, NAL significantly reduced intake of ethanol at the doses of 1,2 and 4 mg/kg intraperitoneally. This effect was particularly evident in "high-preferring" rats (ethanol/total fluid intake > 60%) and was absent in "low-preferring" rats (ethanol/total fluid intake < 30%). Furthermore, a significant correlation ($P < 0.001$) was found between the degree of spontaneous preference (ethanol/total fluid intake ratio) and the reduction of ethanol intake induced by NAL at all doses tested ($r = -0.658$, $r = -0.742$, $r = -0.695$ for the doses of 1,2 and 4 mg/kg respectively). In the second study, Tyr-MIF-1, was ineffective in altering preference for ethanol in the free choice paradigm.

These results further support the hypothesis that the endogenous opiate system plays a role in the rewarding value of ethanol as measured by volitional drinking. The fact that a correlation was present between the degree of spontaneous preference and NAL-induced inhibition, suggests that the proposed genetic predisposition for alcoholism might involve a role for the opiate system.

446.8

THE EFFECTS OF PRENATAL, POSTNATAL AND COMBINED EXPOSURE TO ETHANOL ON LEARNING AND ON HIPPOCAMPAL CELL DENSITY IN WEANLING RATS.

T. Wigal, N.J. Lobaugh and A. Amsel. University of Texas, Austin, TX 78712.

Neonatal rats were exposed to ethanol during postnatal Days 4 to 12 by employing an artificial rearing procedure to administer either 3.0% ethanol (E) or isocaloric maltose/dextrin (C) in a milk formula. These pups were from mothers fed either a liquid ethanol (E) or a control (C) diet on gestational Days 1 to 21. This 2x2 design yielded four treatment groups: CC, CE, EC, and EE.

On postnatal Days 20 to 21 the pups learned an approach response, under either continuous (CRF) or partial (PRF) food reinforcement, which was then extinguished. The partial reinforcement acquisition effect was eliminated in CE, EC, and EE, and the partial reinforcement extinction effect was attenuated in EC and EE and was absent in CE.

Hippocampal cell density was determined at midtemporal level, showing a 12% reduction in CA1 pyramidals and an 11% reduction in mature granules after prenatal exposure to ethanol (EC and EE). The CA4 area was significantly larger after postnatal exposure (CE and EE). There was no effect on immature granule cells. Significant positive correlations were found between extinction slopes after PRF training and CA1 pyramidal-cell density in animals from Groups CC and CE. A significant negative correlation was found between extinction slopes after PRF training and CA4 area in animals from Group EE.

446.9

BIPHASIC EFFECT OF THC ON GLUCOSE UPTAKE IN RAT HIPPOCAMPUS. J. E. Margulies* and R. P. Hammer, Jr. Dept. of Anatomy & Reproductive Biology, Univ. of Hawaii, Honolulu, HI 96822.

Delta-9-tetrahydrocannabinol (THC) causes memory impairment and neurophysiologic alteration in hippocampus. We used the tritiated 2-deoxy-D-glucose (2DG) autoradiographic method to examine the effect of acute administration of THC on regional brain metabolism in hippocampus of male rats. Various doses of THC (0.2, 0.5, 2.0 and 10 mg/kg), solubilized in 5% propylene glycol and 0.5% Tween 80, were injected via intracardiac cannulae followed in 10 minutes by a pulse of 2DG (1.0 mCi/250 g). Animals were decapitated forty-five minutes after 2DG administration. Sham-operated control animals received the same dose of vehicle without THC. THC altered 2DG uptake in a biphasic manner in hippocampus and other limbic structures. The 0.2 mg/kg dose significantly increased 2DG uptake in the stratum lacunosum-moleculare (SLM) of the hippocampus, whereas doses of 2.0 and 10 mg/kg significantly decreased 2DG uptake in the SLM. THC produced differential effects on the pyramidal cell layer and stratum oriens of CA1 and CA2-3. These layers in CA2-3 responded to THC biphasically, however 2DG uptake in these regions of CA1 was unaltered at low doses of THC. Thus, certain regions of the hippocampus are more sensitive to THC. This dose sensitivity may reflect a specific effect of THC as opposed to a nonspecific effect seen at higher doses. Supported by USPHS awards RO1 DA03885 and KO4 NS01161.

446.10

Δ^9-TETRAHYDROCANNABINOL (THC) INHIBITS ARACHIDONIC ACID ACYLATION IN GUINEA PIG CEREBRAL CORTEX SLICES. M. Reichman*, W. Nen*, and L.E. Hokin* (SPON: J.L. Dahl) Dept. of Pharmacology, Univ. of Wisconsin Med. School, Madison, WI 53706

The mechanism of action of THC, the major active ingredient in marihuana, remains unclear. We have found that THC increases unesterified arachidonic acid (AA) levels in guinea pig cerebral cortex slices prelabeled with [^{14}C]AA. We report here that the mechanism underlying this rise involves an inhibition of AA acylation, rather than activation of lipolytic enzymes. The incorporation of AA into brain lipids was measured by incubating cerebral cortex slices with [^{3}H]AA for 1 hr at 37°C without and with THC. The lipids in the tissue were extracted and separated by thin layer chromatography, and the radioactivity in the individual phospholipids and neutral lipids was measured. Treatment with THC concentrations in the range of 2-32 μM elicited dose-dependent and saturable reductions in the esterified [^{3}H]AA levels in membrane lipids. The IC_{50} was on the order of 8 μM, and a maximal reduction in radioactivity of 50% occurred at 32 μM. We observed concomitant rises in the levels of unesterified [^{3}H]AA. The levels of radioactivity were significantly reduced in both the neutral lipid and phospholipid fractions, although the largest decreases in radioactivity were observed in phosphatidylinositol; the levels of [^{3}H]AA in phosphatidylcholine were not significantly affected by THC. Our results indicate that the mechanisms underlying the THC-induced elevation in unesterified AA involve an inhibition in the acylation of membrane lipids, and suggest that inositol-containing phospholipids may play an important role in the mechanisms mediating the response.

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446.11

TOLERANCE STUDIES AND BRAIN NICOTINIC RECEPTORS: A COMPARISON OF CHRONIC NICOTINE TREATMENTS IN RATS. Allan C. Collins and Jeanne M. Wehner. Inst. Behav. Gen., Sch. of Pharm., Univ. of Colorado, Boulder, CO. 80309

Rats were treated for 7 days either with twice daily injections of nicotine (0.8 mg/kg), or by continuous subcutaneous infusion of nicotine (0.8 mg/kg/hr). Behavioral tolerance was assessed using open-field activity and hypothermia. Brain nicotinic receptors were evaluated using ^{3}H-nicotine (NIC) and ^{125}I-a-bungarotoxin (BTX) binding. Chronic injections resulted in tolerance and an up-regulation of NIC binding in cortex and hippocampus, but no change in BTX binding was seen. Tolerance was not lost by 8 days after withdrawal from injections, but levels of NIC binding had returned to normal. After infusion tolerance and an up-regulation of both NIC and BTX receptors was observed in five regions. Tolerance was completely lost and normal levels of NIC and BTX binding had returned in most regions by 8 days after withdrawal from infusion. These results indicate that: 1) continuous infusion produced more substantial changes in NIC and BTX binding and 2) tolerance was longer lasting after chronic nicotine injections than after nicotine infusion. We conclude that the long-lived behavioral tolerance produced after chronic injection may be confounded by other behavioral changes not directly related to nicotine tolerance. (Supported by DA-03194).

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY V

447.1

RECEPTIVE FIELD PROPERTIES AND MORPHOLOGY OF NEURONS IN THE SUPERFICIAL LAYERS OF THE SUPERIOR COLLICULUS OF NEONATALLY ENUCLEATED HAMSTERS. M.M. Nikoletseas, R.D. Mooney and R.W. Rhoades, Medical College of Ohio, Toledo, OH 43699.

Intracellular recording and horseradish peroxidase injection techniques were used to characterize 84 neurons from the stratum griseum superficiale and stratum opticum of 42 hamsters that sustained removal of both eyes on the day of birth. Of the 84 recovered cells, 90.5% (N=76) responded to somatosensory stimulation. These responses were often weaker and receptive fields were more diffuse than for somatosensory neurons recorded in the deep laminae. The vast majority (62.5%) of the recovered neurons were horizontal cells; 12.5% were widefield vertical cells, 3.1% were narrow field vertical cells, 6.3% were stellate cells, and the remainder (15.6%) could not be classified. In the superficial SC laminae of normal adult hamsters (Mooney, R.E. et al. J. Neurosci. 5:2989, 1985), horizontal cells constituted only 13.6% of a sample 59 recovered neurons; 20.3% were widefield vertical cells, 17.0% were narrow field vertical cells, 23.7% were stallate cells, 13.6% were marginal cells, and 11.9% could not be classified. These results suggest that the change in afferent input to the superficial laminae that follows neonatal enucleation may result in either dendritic remodelling or differential survival of neurons with horizontally oriented dendrites. Supported by EY 04170 and BNS 85 00142.

447.2

VIMENTIN AND GFAP EXPRESSION IN DEVELOPING HAMSTER SUPERIOR COLLICULUS. D.Wu*, S.Jhaveri*, K.L.Moya and G.E.Schneider. Dept. Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139. (Spon: J.S. Barlow)

Immunolocalization of vimentin (antibody courtesy of R.McKay) in the neonatal hamster SC reveals a radial pattern of cells fanning out from the ventricular zone. Staining of vimentin-positive cells is observed on postnatal days 1-3 (P1-P3, where P0=day of birth); it is considerably decreased on P5 and P7. However, staining of glial end feet and portions of radial cell processes attached to the pial surface persists at least until P7. A group of labelled cells around the aqueduct of Sylvius shows a tuft of processes that are positioned along the tectal midline. These cells consistently express vimentin even at later stages of development.

Immunohistochemical labelling with an antibody to GFAP (Boehringer), on the other hand, reveals little staining within the SC. The major exception to this is the group of aqueductal cells with processes along the midline, which are intensely GFAP positive. They are co-localized with vimentin-containing cells and may comprise the same population. The GFAP and vimentin-positive processes are in the same position as the "axon-refractory wedge" observed in ultrastructural studies of the tectal midline (see Poston et al., Neurosci. Abs.'88). It is known that destruction of this midline tissue in newborn hamsters which also have one eye removed is sufficient to result in abnormal crossing of the midline by axons from the remaining retina.

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447.3

EARLY DEPRIVATION PRODUCES MOLECULAR AND MORPHOLOGICAL CHANGES IN CAT LATERAL GENICULATE NUCLEUS AND VISUAL CORTEX. A. Guimarães and S. Hockfield. Section of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

Monoclonal antibody Cat-301 recognizes a surface associated antigen on a subset of neurons in the adult mammalian CNS (Hockfield and McKay, 1983, Proc. Natl. Acad. Sci. 80: 5758). In the cat dorsal lateral geniculate nucleus (LGN) Cat-301 expression on Y-cells is sharply reduced by early visual deprivation (Sur et al., 1988, J. Neurosci. 8: 874). In order to further study experience-dependent alterations in the expression of molecular species in visual pathways, new born Balb/c mice were injected with homogenized, dissected LGN from dark-reared cats to induce an immunosuppression of antigens present in deprived tissue, and were immunized with normal cat LGN to elicit an immune response selectively to experience-dependent antigens. Monoclonal antibody Cat-304, which recognizes a surface associated antigen on neuronal cell bodies and proximal dendrites, was generated. In normal cat LGN, Cat-304 labels cells in laminae A, A1 and C, in interlaminar zones and in the medial interlaminar nucleus. In dark-reared cat LGN the number of labelled cells is markedly reduced. In cortical area 17, Cat-304 positive cells are densely distributed in two bands, in layers IV and VI. Labelled cells are also present in layers II, III and V. In area 17 of dark-reared cats, the number of antibody positive cells is reduced. The loss of antibody positive cells is most pronounced in layer VI, but layers II, III and V also contain fewer stained neurons than normal. The reduction in expression of Cat-304 antigen in dark-reared cat visual cortex is paralleled by that of Cat-301. Double-label experiments show that Cat-304 and Cat-301 recognize distinct sites on the same cell populations in LGN and area 17. These results suggest that the expression of an antigen (or antigens) recognized by Cat-304 and Cat-301 is mediated by visual experience from birth.

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447.4

POSTNATAL DEVELOPMENT OF NEURONS AND OPTIC TRACT AXON ARBORS IN THE HAMSTER DORSAL LATERAL GENICULATE NUCLEUS: IN VITRO HRP STUDY. P.G. Bhide*1,2, A.R. Lieberman*2 and D.O. Frost1. 1Sect. Neuroanat., Yale Univ., New Haven, CT 06510 and 2Dept. Anat., Univ. Coll. London, WC1E 6BT.

Neurons and optic tract (OT) axons (including terminals) in the dorsal lateral geniculate nucleus (LGd) were labeled with HRP in brain slices and their morphology analyzed. On the day of birth (P0), the majority of labeled neurons resemble immature projection neurons and some resemble immature interneurons (e.g. Parnavelas et al., 1977, J. Comp. Neurol. 171:481). Their processes bear growth cones at the tips and on the trunks and are predominantly oriented towards OT. Some transiently invaginate and form synapses with OT axons and may induce the latter's collateralization. During the third week, growth cones disappear and mature dendritic patterns emerge.

The OT has 2 components: superficial optic tract (SOT) axons form a sheet on the surface of LGd, whereas fascicles of internal optic tract (IOT) axons course through LGd, parallel to SOT. In adults, only SOT axons have thalamic collaterals (Schneider & Jhaveri, 1983, Neurosci Abs. 9:809). On P0, IOT axons emit transient collaterals to the ventrobasal complex (Langdon, et al., 1987, Neurosci. Abs., 13:1023). At this stage, SOT axons are largely unbranched. A few emit collaterals restricted to the superficial LGd. Such collaterals proliferate and reach the deep border of LGd by the end of the first postnatal week. During the second week, SOT collaterals begin to form arbors which, towards the fourth week, resemble those in the adult. Thus, the development of OT axons is characterized by 3 modes of growth: elongation, collateralization and arborization

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447.5

MONOCULAR ENUCLEATION AT BIRTH ALTERS THE DISTRIBUTION OF DISTINCT TYPES OF RETINO-GENICULATE TERMINALS IN THE HAMSTER. R.S. Erzurumlu, S. Jhaveri* and G.E. Schneider. Dept. Brain & Cognitive Sciences, M.I.T., Cambridge, MA, 02139.

The distribution of morphologically distinct retinogeniculate terminals was analyzed in adult hamsters which had one eye removed on postnatal day 1. Retinal terminals were labeled with HRP implants in the optic tract (OT) ipsilateral or contralateral to the remaining eye.

Normally there is a segregation of R1 and R2 terminals within the dorsal nucleus of the lateral geniculate body (LGBd) (see Jhaveri et al., Neurosci. Abs. 1988). Neonatal enucleation results in partial atrophy of the LGBd and an expansion of the territories occupied by the remaining eye. Contralaterally, the deafferented ipsilateral projection zone is occupied by R1 and R3 terminals only. Large numbers of R1 terminals are also found in the superficial zone, where they normally would not be present. Furthermore, R2 terminals, normally located superficially, now form abnormally large clusters and extend deeper into the nucleus. Ipsilateral to the remaining eye, the expanded retinal projection is comprised of R1 and R3 terminals only. In these cases R1 terminals are seen subjacent to the OT, a location normally occupied by R2 terminals from the contralateral eye. R2 terminals, on the other hand, are not present in the expanded ipsilateral projection .

These results indicate that morphology of retinofugal axon terminals may be dictated by retinal ganglion cell specificity and survival rather than by their target environment. The altered distribution of different terminal types within the LGBd, following neonatal monocular enucleation, also suggests an anomolous convergence of inputs from different types of retinofugal axons onto geniculate neurons.

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447.6

ABNORMALLY PROJECTING RETINAL AXONS DISPLACE GAP-43 POSITIVE TERMINALS IN THE LATERAL POSTERIOR NUCLEUS OF THE THALAMUS. K.L.Moya, L.Carman*, L.I.Benowitz and G.E.Schneider. Dept. Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139 and Dept. of Psychiatry, Harvard Med. Sch., McLean Hosp., Belmont, MA 02178.

Lesions of the superior colliculus (SC) in neonatal hamsters are followed by regeneration of the damaged retinal ganglion cell axons over the lesioned area and the formation of abnormal, dense patch-like retinal projections in the LGN and LP. Immunocytochemical localization of GAP-43, a membrane phosphoprotein associated with neuronal growth, using a monospecific antibody has revealed that the LP shows relatively heavy staining in normal adult hamsters. We have found that in animals which had received an early lesion of the SC, GAP-43 staining was excluded from the areas of the LP that received retinal input, while the areas of LP that did not receive retinal imput showed the normal high levels of GAP-43. The source of GAP-43 positive terminals remains to be determined: lesions of the posterior cortex or SC do not diminish GAP-43 staining in the LP. In addition, shortly after ibotenic acid lesions of the LP, overall levels of GAP-43 were not reduced.

These results show that retinal ganglion cell axons, which lose their GAP-43 positive staining in the early postnatal period, do not persist in the expression of GAP-43 even when induced to project into an area normally high in this protein. Furthermore, it appears that the retinal terminals may displace GAP-43 positive axon terminals resulting in the abnormal GAP-43 negative areas in LP after early SC lesions.

[Supported by NSF, NEI, NINCDS.]

447.7

MORPHOLOGICAL MATURATION OF IDENTIFIED PROJECTION NEURONS IN THE CAT'S LATERAL GENICULATE NUCLEUS (LGN). D. Raczkowski, C.-S. Lin, and W.L. Salinger. Dept. of Neurobiol., Duke Univ., Durham, NC 27710 and Dept. of Psychol., Univ. of North Carolina, Greensboro, NC 27412.

In these experiments, we examined how the adult structure of geniculocortical neurons develops by injecting rhodamine microspheres into the visual cortex of early postnatal and adult cats and, subsequently, viewing brain slices of the LGN with epi-fluorescent microscopy so that retrogradely labeled neurons could be injected intracellularly with FITC-HRP. In the adult brain slice, all three classes (1, 2, and 4) of geniculate projection neurons were recognized. In the neonate (kittens 3-5 weeks of age), neurons resembling each morphological class also could be identified. Although many class 1 and class 4 geniculate cells in the central representation of the visual field possess adult-like dendritic arbors at these ages, most class 2 neurons do not. Despite these differential rates of maturation, geniculate cells in every class at these ages exhibit morphological features not seen in the adult. These include more proximal dendrites, an increase in the number and assortment of dendritic appendages and, occasionally, exuberant intrageniculate axonal arbors. These transitory dendritic features suggest that developing neurons in the LGN actively participate in the acquisition and maintenance of synaptic interactions with their inputs. Supported by EY06951, NSF8519709, NIMH04849, and NSF8606570.

447.8

ANALYSIS OF SYNAPTOGENESIS USING MULTIPLE ULTRASTRUCTURAL CRITERIA IN THE RAT VISUAL CORTEX AND SUPERIOR COLLICULUS. B.W. Bakkum, L.A. Benevento and R.S. Cohen. Dept. of Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL 60612.

We examined the developmental patterns and time course of synaptogenesis in the developing rat visual cortex (VC) and superior colliculus (SC). The synaptic parameters measured have already been implicated as morphological correlates of plasticity but, taken together, have not been extended to deprivation studies. The VC, SC, and as a control, the temporal cortex of Sprague-Dawley rat pups, raised either in 14 hrs light/10 hrs dark or in total darkness, were fixed at postnatal days (PND) 7, 14, 21 and 28 and processed for EM analysis according to standard procedures. In the SC of light/dark-reared animals the number of synapses increased up to PND-21 and then declined. Other parameters which follow the same developmental pattern are positively curved and straight synapses, asymmetrical, dendritic, and spinous synapses. However, symmetrical synapses and negatively curved synapses peaked earlier at PND-14 and then also declined. In the VC the number of synapses at PND-7 was about equal to that found in the SC. This number also increased up to PND-21, but the number of synapses/unit area was twice as great as in the SC. This number declined to adult levels by PND-28. Asymmetrical, straight, and axospinous synapses follow a similar pattern. Symmetrical and negatively curved synapses peak at PND-14 before declining, whereas positively curved synapses increase to PND-28. There were low numbers of perforated postsynaptic densities and axosomatic synapses in the VC and SC at all stages. Dark-reared data suggest that there is little influence of sensory deprivation on the developmental patterns (i.e., trends) and time course of the aforementioned synaptic parameters. Supported by NIH Grant NS 15889.

447.9

EARLY MONOCULAR DEPRIVATION AND RAT BRAIN VISUAL SYSTEM 2-DEOXYGLUCOSE UPTAKE. R.M. Cooper and A. Jeeva.*
Psychology Dept., Univ. Calgary, Calgary, AB, T2N 1N4.

Hooded rats had one eye lid-sutured (MD) for 6 wk beginning at age 10 d. The rats were tested by binocularly exposing them to a display of stripes after the 2-DG injection. Autoradiographic analysis revealed that 2-DG uptake was greater in the LGN, VC and SC in the hemisphere lying contralateral to and primarily fed by the deprived eye. This "sensitization" effect has also been reported for dark-reared rats (Toga, A.W., Dev. Brain Res., 37: 209, 1987). Similar interhemispheric differences in 2-DG uptake, even in VC, arose when MD rats were tested with light-diffusing eye occluders. Thus the increase in 2-DG uptake may be attributed to a reduction in receptive-field specificity--normally, diffuse light has little or no effect on rat VC 2-DG uptake. But decreases in glucose utilization also occur after MD. In rats which had undergone MD for 12 wk the sensitization effect was confined to the more monocular portion of the contralateral LGN, while in the more binocular portion, 2-DG uptake was reduced. This same pattern of LGN uptake was also observed in 6 wk MD rats which were allowed 4 wk of "recovery." Thus, the deprived eye loses control in binocular regions and this process can continue even after sight is restored.

447.10

EFFECT OF MONOCULAR LID SUTURE, ENUCLEATION, AND RETINAL IMPULSE BLOCKAGE ON CYTOCHROME OXIDASE REACTIVITY IN LAMINAE II-III OF MACAQUE STRIATE CORTEX. T. C. Trusk, M. Wong-Riley, and W. Kaboord*
Dept. of Anatomy & Cellular Biology, Med. Col. of WI, Milwaukee, WI 53226.

We sought to quantify the size and numerical density of cytochrome oxidase-reactive and nonreactive neurons in control and experimental supragranular puffs (CP & EP) and intra control and experimental interpuffs (ICI & IEI) of monkeys subjected to monocular lid suture (MD), enucleation (ME), or retinal impulse blockage with tetrodotoxin (TTX). Nembutal anesthesia (30 mg/kg) was used during surgery and euthanasia. We found no cell loss in all experimental monkeys. The average size of reactive cells in CP and ICI were similar to those of normal monkeys. Numerical densities of reactive neurons were decreased in EP and IEI of all experimental monkeys. Their average size was reduced in MD and TTX. However, in a long term ME, the sparse population of remaining reactive cells were mainly medium and large in size. The average size of nonreactive neurons in EP remained similar to that found in CP. In contrast, they were consistently smaller in IEI than in ICI, and this difference was significant in adult and juvenile MD's (11 to 48 weeks). Optical densities (OD) of EP and IEI were consistently lower than those of CP and ICI. This depression was less severe in adult monkeys lid sutured for 11 weeks or given intravitreal TTX for 2 weeks; and was most severe in an adult treated with TTX for 4 weeks. Optical density of CP was within the range found in normal monkeys, while OD in ICI was consistently greater, and OD in IEI was lower than normal interpuffs. These results suggest that: a) metabolically active neurons of laminae II-III are more sensitive than less active cells to sensory deprivation in adults; and b) there is an increase in metabolic activity within the interpuff region innervated predominantly by the spared eye. [Supported by NIH EY07016 (TCT) and EY05439 (MWR).]

447.11

EFFECTS OF VISUAL DEPRIVATION ON DENDRITIC MATURATION OF SPINY STELLATE NEURONS IN MONKEY PRIMARY VISUAL CORTEX. J.S. Lund and S.M. Holbach*. Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

Dendritic field extent and spine number have been compared for neurons in V1 visual cortex of age-matched visually deprived and normal macaque monkeys. Golgi rapid impregnated spiny stellate neurons from thalamic recipient layers 4Cβ and 4Cα were compared for dendritic arbor size, using a Sholl ring intersection method, and for spine number over 10μm segments taken at 20μm intervals from soma to dendritic tips; an average total spine coverage per neuron was then calculated. Rearing to 2 months with bilateral eye-lid suture from 3 days of age produced no significant difference from normal in total spine coverage per neuron. Rearing in the dark from 13 days to 3 months of age produced a 38% greater than normal total spine coverage in β but little change in total spine population in α. Rearing in the dark from 10 days of age to 6 months produced a higher than normal total spine coverage in both α (+58%) and β (+28%). Returning an animal to the light for 6 weeks after dark rearing from day 9 to 6 months produced near normal total spine coverage in α but higher than normal spine coverage (+46%) in β. Our conclusion is that visual deprivation induces a retardation in normal spine and dendritic arbor loss during the early postnatal period which is more readily reversed in α than in β by restoring normal visual input. Supported by NEI grant EY05282.

447.12

DEVELOPMENT OF LATERAL SUPRASYLVIAN VISUAL CORTICOTECTAL PROJECTIONS IN NEONATAL CATS. L.L. Bruce, J.G. McHaffie, and B.E. Stein. Dept. Anatomy, Creighton University, Omaha, NE 68178 & Dept. Physiology, Medical College of Virginia, Richmond, VA 23298.

To study the maturation of the projections from the medial bank of lateral suprasylvian (LS) sulcus to superior colliculus (SC), fast blue (FB; a long-lasting tracer) was injected into the SC at one day postnatal (dpn). At 27 dpn a nuclear yellow (NY) injection was made that encompassed the first site. Medial bank neurons that projected to SC at birth (i.e. FB labeled) were present homogeneously throughout layer V. Far fewer neurons maintained their projections at 27 dpn (i.e. double-labeled) but these had the same distribution as the FB-labeled neurons. Neurons labeled only with NY were rare.

The possibility that neurons losing corticotectal projections had 'inappropriate' targets in SC, was also evaluated. Injections of tritiated leucine were made in the medial bank of LS at 0 and 14 dpn. The terminal labeling pattern indicated that at birth, as in adulthood, the medial bank terminates topographically in the superficial laminae and dorsal aspect of the intermediate laminae of the ipsilateral SC. Therefore, although corticotectal connections from medial suprasylvian cortex are lost during maturation, their loss (like those from striate cortex) reflects refinements within the mature target zone in the SC.

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447.13

VISUAL FUNCTION ALTERATIONS RESULTING FROM NEONATAL CORPUS CALLOSUM SECTION ARE LINKED TO PRIMARY VISUAL CORTEX. A.J. Elberger. Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Col. of Med., Memphis, TN 38163.

Previous studies of cats with neonatal corpus callosum section have linked behavioral alteration of perimetric visual field extent (Elberger 1979) to primary visual cortex (Elberger and Spydell 1985). When physiological studies found visual functional alterations at the cellular level, primary visual cortex was involved (Elberger 1981; Elberger and Smith 1985) while lateral suprasylvian cortex was not (Elberger and Smith 1983).

In cats with corpus callosum section at 1-6 postnatal weeks of age, three different kinds of measurements of visual function were obtained from each cat: 1)behavioral measurement of monocular and binocular visual acuity; from single striate cortical cells, physiological measurement of 2)spatial frequency threshold and 3)ocular dominance. All three measurements were significantly related to the age at which the corpus callosum section occurred, and the greatest deficits in visual function occurred in the youngest callosum sections. Compared with the results from normal cats, only the cats with callosum section during postnatal weeks 1 and 2 showed significant deficits in all measurements. From individual cats' data the three visual functions were found to be significantly interrelated. The results support the hypothesis that the most significant effect of neonatal corpus callosum section is to alter the functional capabilities of primary visual cortex. EY06362

447.14

EFFECTS OF ALTERNATING MONOCULAR OCCLUSION ON MATURATION OF FELINE VISUAL CALLOSAL CONNECTIONS. D.O. Frost[1], Y.P. Moy[1]* & D.C. Smith[2]. [1]Sect. of Neuroanatomy Yale Univ. Sch. Med., New Haven, CT 06510 and [2]Dept. of Psychology, Southern Illinois. Univ., Carbondale, Ill. 62901

In cats, neurons projecting through the corpus callosum (callosal neurons; CN's) fill areas 17 & 18 at birth; during the first 3 months of life, they narrow their distribution to the region of the 17/18 border. Rearing with strabismus (S), monocular enucleation (ME) or monocular eyelid suture (MD) stabilizes some normally transient CN's in medial area 17. Why do S, ME and MD similarly affect callosal development? Rearing with S, ME or MD all cause most neurons in areas 17 & 18 to respond predominantly to stimulation of one eye; in normal cats, most of these neurons respond well to stimulation of either eye. To test the relationship between loss of binocularity and stabilization of transient CN's, we reared cats with alternating monocular occlusion (AMO), which also makes most area 17/18 neurons monocular, but unlike S, ME and MD, lacks other physiological effects. 4 cats were raised in total darkness except that 6 d/wk they explored a normally lit room with one eye occluded; each eye was occluded on alternate days. Visual experience was gradually increased from 1 to 8 h/d. 48 h before sacrifice at age 3 months, cats received unilateral HRP injections that filled the posterolateral and lateral cortical gyri. The distribution of retrogradely labeled CN's in the opposite cortex was determined on computer reconstructions from serial, TMB-processed sections. 6 normal, adult cats were similarly treated. In AMO-reared cats, as in cats reared with S, ME or MD, normally transient CN's in medial area 17 were stabilized. This suggests that experience-dependent loss of cortical binocular responsiveness and stabilization of normally transient callosal connections are related but a causal relationship remains uncertain. Supported by EY-03465 from NIH & 5-417 from March of Dimes.

447.15

VISUAL CALLOSAL PROJECTIONS IN THE STRIATE CORTEX OF THE RABBIT FOLLOWING NEONATAL BINOCULAR ENUCLEATION. A.M. Grigonis and E.H. Murphy. Dept. of Anatomy, Hahnemann University, Phila., PA 19102, and Dept. of Anatomy, Medical College of Pennsylvania, Phila., PA 19129.

The organization of cells which comprise the projection of the corpus callosum (CC) of the rabbit are exuberant at birth and normally retract during development to become restricted to the 17/18 border in adults. Monocular enucleation (ME) on the day of birth results in an exuberant CC cell distribution in the adult similar to that observed in the neonate. Dark-rearing results in a CC cell distribution which is more sparse than normal and which extends into the medial portion of area 17 further than in the normal adult rabbit but not as far as in the neonate or the ME rabbit. In the present study we examined the effects of neonatal binocular enucleation (BE) on the CC cell distribution. Seven Dutch-Belted rabbits had BE on the day of birth. After reaching adulthood, multiple injections of HRP (Sigma VI, 20% in H_2O) were made (15 µl) throughout one entire visual cortex. Animals were perfused 24 hours later and the brains were cut and reacted with TMB. BE rabbits had a sparse CC cell distribution similar to that observed after dark-rearing. However, the CC cell distribution was not exuberant. In addition, there were fewer cells in the infragranular layers of BE rabbits compared to either normal, ME, or dark-reared rabbits. The results of the present study provide further evidence that visual experience and the presence of the primary afferent visual pathway make different contributions to the postnatal development of the corpus callosum. Supported by NIH grants EY06986 and EY02488.

447.16

DEVELOPMENT OF VISUAL THALAMOCORTICAL PROJECTIONS IN THE FETAL RAT. B.S. Reinoso and D.D.M. O'Leary, Dept of Neurosurgery & McDonnell Ctr for Studies of Higher Brain Function, Washington Univ Sch Med, St.Louis, MO 63110.

The cortical ingrowth of visual thalamic axons was studied by postmortem DiI labeling in E17-20 rats. Embryos were perfused with 10% formalin, their brains removed, transected just rostral to the colliculi, and DiI crystals placed in the region of dorsal lateral geniculate nucleus. The brains were stored in 2% formalin at 37° for 6-10 days. Sections were cut and analyzed with fluorescence and Nomarski optics. The DiI uptake site is confined to caudal dorsolateral thalamus. At all ages, anterograde labeling is most dense in the caudal portion of the thalamic radiation. Labeled axons are present in the intermediate zone (IZ) of fronto-parietal regions by E17 and within the occipital area by E19. They accumulate in more superficial IZ, but some extend into deep IZ and even into the proliferative zone. Initially, labeled axons invade deep layers of cortical plate in rostral regions, occurring on E18; this invasion then proceeds caudally. The labeled axons form branches along their trajectory; many extend out of the IZ. The presence of visual thalamic afferents to non-visual cortical areas is evidence of a transient exuberancy of thalamocortical projections. We are correlating these results with cortical neuronogenesis in mature littermates labeled with 3H-TdR in utero 2 hrs before removal of the fetuses used here for DiI labeling.

447.17

VISUAL CORTEX TRANSPLANTED TO FRONTAL REGION FORMS RECIPROCAL THALAMIC AND CALLOSAL CONNECTIONS TYPICAL OF MOTOR CORTEX. Dennis D.M. O'Leary, Dept of Neurosurgery & McDonnell Center for Studies of Higher Brain Function, Washington Univ Sch Med, St. Louis, MO 63110

In an attempt to define further the influence that the position of a developing cortical neuron in the tangential plane of the neocortex has on the connections that it will establish, pieces of E17 fetal occipital cortex, exposed to ^{3}H-thymidine in utero on E16, were transplanted to the frontal region of newborn rats. The host rats were allowed to mature (P35 to 6 mo); WGA-HRP was then iontophoresed into the transplant. 1-2 days later, the rats were perfused and their brains frozen sectioned. Alternate sections were processed for TMB histochemistry or autoradiography. In 3 of 17 rats, the injection is confined to the transplant, the borders of which can be delineated from autoradiograms. In these cases, anterograde label and retrogradely filled neurons are found within thalamic nuclei that normally have connections with motor cortex, and in the region of contralateral cortex homotopic for the location of the transplant in the host cortex. No labeling is seen in visual thalamic nuclei, nor in regions of cortex normally connected with occipital cortex. These results indicate that heterotopic neocortical transplants establish permanent thalamic and callosal connections typical of their new site in the host cortex, not of their origin.

447.18

GENERATION OF SPECIES DIFFERENCES IN EYE AND BRAIN CONFORMATION: ALLOMETRIC AND NEUROGENETIC STUDIES. G.M. Perez, D. R. Sengelaub, K. C. Wikler, and B.L. Finlay, Dept. of Psychology, Cornell University, Ithaca NY 14853

The Syrian hamster (Mesocricetus auratus) and the Mongolian gerbil (Meriones unguiculatus) are two closely related species that differ substantially in their retinal topography, visual acuity and developmental time course. The gerbil has a greater number of retinal ganglion cells, a greater center-periphery ratio of those cells, and longer gestation and maturation. In this study we have investigated the relationship between neurogenesis and allometric measurements of eye, brain and body size.

These allometric measures were compared from the initiation of neurogenesis to adulthood (hamster n=30; gerbil n=30). The body weight of gerbils is always less than hamsters. Brain size, however, is nearly identical although the duration of neurogenesis is substantially longer in gerbils. Similarly, eye size is nearly identical at the end of an extended period of neurogenesis that produces about twice as many retinal ganglion cells in the gerbil. The gerbil's eye is initially ovoid, longer on its nasotemporal axis, while the hamster eye is spherical. The gerbil eye through extended growth becomes larger and spherical which may directly contribute to the development of an asymmetric visual streak in gerbils. Thus both initial shape and developmental heterochrony contribute to the development of these species' differences. Supported NIH grants RO1 NS19245 and KO4 NS00783

TROPHIC AGENTS VI

448.1

EFFECT OF NGF ON TYROSINE HYDROXYLASE mRNA AND EXTRACELLULAR MONOAMINES LEVELS IN PRIMARY CULTURES OF RAT ADRENAL CHROMAFFIN CELLS. D. G. Roufa, D. R. Studelska* L. M. Pullan, S. R. Rapp*, V. W. Engleman* and **K. L. O'Malley. G. D. Searle/CNSDR, St. Louis, MO 63198 and **Anatomy/Neurobiology Dept. Washington University School of Medicine, St. Louis, MO 63110.

A subpopulation of adrenal medullary cells, obtained from neonatal rats (P7) and grown in dissociated cell culture, express NGF receptors (NGFR) as determined by immunocytochemical localization of NGFR specific mAb 192. Colocalization of NGFR with either tyrosine hydroxylase (TH) specific antibodies or TH mRNA, using in situ hybridization with ^{35}S-RNA probe, revealed two distinct cell populations. One population was both NGFR and TH positive and the other was only NGFR positive. All TH positive cells were also NGFR positive and both populations were NGF-responsive. Cells cultured in medium containing NGF had twice as much TH message (F=62.99, dt=1/100, P<0.0001) as cells grown in the absence of NGF. This increase was evident in cells from male and female animals. There was no significant effect of sex on TH message expressed and no indication of a treatment x sex interactions. Quantitative evaluation of monoamine neurotransmitters and their metabolites present in the culture medium conditioned by the adrenal medullary cells was performed using HPLC-EC system. The level of monoamine neurotransmitters and their metabolites was significantly altered when adrenal medullary cells were grown in the presence of NGF.

448.2

NGF REGULATES THE EXPRESSION OF PREPROTACHYKININ AND CGRP GENES IN ADULT RAT SENSORY NEURONS. R.M. Lindsay and A.J. Harmar, (SPON: M.Gaze), Cell Biology, Sandoz Institute for Medical Research, London, England, and MRC Brain Metabolism Unit, Edinburgh, Scotland.

Although not requiring nerve growth factor (NGF) for survival (RML, J.Neurosci., in press, 1988), adult rat dorsal root ganglion (DRG) neurons cultured with NGF show elevated levels of the neuropeptides Substance P (SP) and calcitonin gene-related peptide (CGRP) (Lindsay et al., 1987, Neurosci.Abstr. 13, 518). To confirm that the effect of NGF is due to an increase in the rate of synthesis of these two peptides, we have studied the effects of NGF on the expression of the genes encoding their precursors. After culture for periods between 4 hours - 5 days with or without NGF, total cellular RNA was isolated by incubation of adult rat DRG cultures (20-50,000 neurons) with SDS/Proteinase K followed by phenol/chloroform extraction. Messenger RNAs coding for the precursors to SP (preprotachykinin:PPT; Harmar et al., FEBS Lett. 208, 67-72, 1986) and CGRP were quantitated by northern blot analysis. In cultures deprived of NGF, PPT and CGRP mRNA levels declined with time in culture. In NGF-treated cultures, increased levels of both mRNAs were detectable within 24h and after 5 days levels of PPT and CGRP mRNA were over 20-fold higher than in control cultures. Even when cultures were initially deprived of NGF for 5 days, similar increases in the levels of both mRNAs were achieved upon NGF addition, indicating that the effects of NGF are not mediated through enhancing survival of PPT and CGRP-producing cells. These results suggest that NGF may exert a continuous trophic influence upon mature sensory neurons through regulation of specific neuronal functions such as the expression of certain neuropeptides, as shown here.

448.3

EARLY GENE REGULATION BY NERVE GROWTH FACTOR: INDUCTION OF AN INTERFERON-LIKE GENE. F.Tirone and E.M.Shooter, Department of Neurobiology. Stanford University, School of Medicine, Stanford, CA. 94305.

Nerve growth factor (NGF) is a neurotrophic molecule responsible for the maintenance and development of sympathetic and some sensory neurons in vertebrates. NGF also induces chromaffin cells, a neural crest derived tissue, to differentiate into sympathetic neurons, with a transcription-dependent mechanism. In order to gain some insight into the early steps of this differentiative process, we made a cDNA library from PC12 cells (a line derived from a chromaffin cell tumor) one hour after treatment with NGF. We identified four cDNA clones (Tirone, F., and Shooter, E.M.Soc.Neurosci.Abstr.,13(1):551,1987), PC1, PC2, PC3 and PC4, corresponding to different mRNA species highly induced by NGF as well as by epidermal growth factor and cyclic AMP (with the exception of PC4). Sequence analysis showed that PC1 corresponds to NGFI-A, recently described as an NGF-inducible cDNA coding for a transcriptional regulatory factor, while PC4 is homologous to the partial sequence of a putative mouse β interferon (Skup, D., et al., Nucleic Ac.Res. 10:3069, 1982). We cloned a full-length cDNA copy of PC4 and found that its deduced protein sequence, 449 amino acids long, is also significantly related to the entire sequence of the rat γ interferon protein. The analysis of expression in rat tissues showed that PC1, unlike the other mRNAs, is present in the adult rat brain, beginning as early as 7 days after birth, in a period related to growth and differentiation of the glial cells. PC2, PC3 and PC4 mRNAs are instead expressed in placenta and amnion - tissues rapidly proliferating and differentiating - and in the neural tube (with the exception of PC2) between 12 and 14 days of gestation, in a period related to neuroblast proliferation and, in part, differentiation. An increase (for PC1) or a decrease (for PC4) of expression was also observed during differentiation of the mouse myoblast cell line C2C12. These results suggest that the four clones could have a role in some differentiative and/or proliferative events, triggered by NGF or by other factors in the brain or in non-neuronal tissues. PC4 could exert this action, in analogy to that of interferons and lymphokines (regulators of cell proliferation and differentiation) by influencing gene expression at levels (e.g. RNA stability) other than RNA transcription (as could be for PC1).

448.4

PHORBOL MYRISTATE ACETATE (PMA) AND NERVE GROWTH FACTOR (NGF) HAVE SIMILAR EFFECTS ON ADRENAL CHROMAFFIN CELLS IN PRIMARY CELL CULTURE. M.A. Herman*#, C.A. Schulz*, I. Parada*† and P. Claude*. Wis. Reg. Primate Res. Ctr., #Dept. of Physiology and *Neurosciences Training Program, University of Wisconsin, Madison, WI, 53715.

NGF promotes survival, neuritic outgrowth and proliferation of cultured chromaffin cells from young rats (Lillien and Claude, 1985). PMA, which activates protein kinase C (PKC), mimics the action of diacylglycerol, a second messenger molecule involved in the action of some hormones (Nishizuka, 1986). PMA has been reported to mimic the effects of NGF in primary cultures of neurons (Montz et al., 1985; Wakade and Wakade, 1986). We have examined the effects of PMA, alone or in the presence of NGF, on chromaffin cells grown in dissociated cell culture for 6 days or more. Cultures were grown with or without PMA (30 ng/ml; Sigma) or NGF (10 or 100 ng/ml 7S NGF; Collaborative Research) on collagen-coated substrata in Medium 199 + 20% charcoal-stripped fetal calf serum, then scored for the proportion of cells bearing neurites or labeled by a 24 hr exposure to ^{3}H-thymidine. Some were processed for immunocytochemistry or scanning EM. **Results:** Long-term incubation with PMA produced effects similar to those produced by NGF. PMA increased cell number and proliferation, and elicited the outgrowth of neurites, although they were fewer in number and morphologically different from those elicited by NGF. In PMA + NGF, as compared to NGF alone, neuritic outgrowth was enhanced while cell proliferation was reduced, as was also observed when PMA was added for the last 2 days of a 6-day incubation in NGF (Lillien and Claude, in prep.). Cells from animals of different ages responded similarly to PMA. Thus, PMA, possibly acting through PKC, mimics or modulates long-term effects of NGF.

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448.5

DEVELOPMENTAL EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR (NGF-R) mRNA IN HYPERTENSIVE AND NORMOTENSIVE RATS. K.A. Curto*, R.E. Stitzel*, R.J. Head*, J.S. Strobl*. (SPON: C.R. Craig) Dept. of Pharmacology & Toxicology, West Virginia University, Morgantown, WV 26506

NGF receptor mRNA levels were measured to examine the role of nerve growth factor receptor gene expression in the etiology of hypernoradrenergic innervation in the genetically spontaneously hypertensive rat (SHR). Total heart (H), aorta (A) and mesenteric artery (M) RNA was extracted from 10, 60 and 100 d SHR and normotensive Wistar Kyoto rats (WKY) and hybridized to P^{32}-NGF-R riboprobe. The degree of sympathetic innervation in the adult rat is A < H < M. Expression of NGF-R was developmentally regulated in all 3 tissues examined. Levels in M and A were 10-fold greater in 10 d than in the 60-100 d rats. Highest NGF-R levels in 10 d rats were seen in H and fell 2 to 3-fold at 60-100 d. Absolute levels of NGF-R mRNA in M and A of both rat strains, and in WKY H were the same in the 10 d rats. The NGF-R content in 10 d SHR H and M was 2 times that in WKY rats, but was not statistically significant.

Thus, NGF-R mRNA levels at the ages tested do not correlate with the degree of sympathetic innervation seen in the adult. Independent assays from this laboratory showed that NGF mRNA in M is 5 times greater in the 10 d SHR than WKY. Hyperinnervation of SHR M therefore correlates with elevated expression of NGF, but not NGF-R gene expression. (Supported by NIH grant HL 36885).

448.6

DISTRIBUTION OF PRO-NERVE GROWTH FACTOR-LIKE IMMUNOREACTIVITY (PRO-NGF-LI) IN THE ADULT RAT BRAIN. M.C. SENUT*, E. DICOU*, Y. LAMOUR and P. BRACHET (SPON: S. DROUVA).U161, 2, rue d'Alesia 75014 PARIS and INSERM U298, CHR, 49033 ANGERS, FRANCE.

In the adult rat, the localization of the pro-NGF has been studied in various brain and spinal cord regions, using affinity-purified sera raised against three synthetic peptides that reproduce epitopes of the pro-NGF, and immunohistochemical (IHC) methods. The three antibodies labeled similar regions, but each sera resulted, for a given structure, in a specific distribution pattern. Strong pro-NGF-LI was present in neocortex, medial septum, hippocampus, globus pallidus, some thalamic nuclei, olfactory bulb, reticular nuclei of the brain stem, whereas lateral septum, hypothalamic nuclei, cerebellum, substantia nigra, motoneurons of the ventral horns, the intermediolateral columns and dorsal horns cells showed less intense labeling. The pro-NGF-LI was mainly observed within cell bodies but some immunoreactive fibers were noticed in corpus callosum, commissura anterior, capsula interna and spinal fasciculi. To identify the pro-NGF-LI cell bodies, the IHC procedure was combined with the retrograde transport of a WGA-apoHRP-Gold complex (Basbaum and Menetrey '87) injected in thalamus, hippocampus and spinal cord; results provide evidence that the pro-NGF-LI is localized within neuronal cell bodies. Studies using antibodies against β-NGF are in progress.

448.7

NERVE GROWTH FACTOR ENHANCES SURVIVAL OF IDENTIFIED PROJECTION NEURONS IN THE RAT SEPTAL AND DIAGONAL BAND REGIONS IN VITRO. Y. Arimatsu*, M. Miyamoto*, H. Tsukui* and H. Hatanaka*. (Spon: T. Hama). Mitsubishi Kasei Inst. of Life Sci., Tokyo 194, Japan.

It has been suggested that nerve growth factor (NGF) enhances survival of cholinergic neurons in basal forebrain both in vivo and in vitro, as judged from histochemical observations for AChE and ChAT. However, previous studies did not completely rule out a possibility that NGF enhanced the expression of these enzymes and not the survival of the neurons. We here support the possibility of neuronal survival by applying NGF in vitro on rat basal forebrain neurons that had been retrogradely labeled in vivo.

Rats of 4 days of age were injected bilaterally into the hippocampus with fluorescent latex microspheres. After 20 to 24 hrs, tissue fragments were dissected out from the medial septum (MS) and vertical limb of the diagonal band (vDB) and treated by papain and DNase. The dispersed cells were cultivated for 3 days with or without 100 ng/ml 2.5S NGF. The number of cells labeled with fluorescent latex microspheres was counted under a fluorescence microscope. Some of the cultures were stained for AChE by a modified Koelle's method, and the others were stained for GABA-immunoreactivity using rabbit anti-GABA antiserum.

The number of fluorescent latex microsphere-labeled neurons in culture with NGF was much greater than that without NGF. The result clearly indicates that NGF enhances the survival of MS and vDB neurons projecting to the hippocampus. Additionally, it was shown that NGF enhanced the survival of both AChE-positive and AChE-negative neurons, and also, the survival of both GABA-positive and GABA-negative neurons. These results suggest that NGF enhances survival of not only cholinergic neurons but also GABAergic projection neurons in MS and vDB.

448.8

INJURED SENSORY NEURONS RESPOND TO DELAYED INFUSION OF NERVE GROWTH FACTOR. V.M.K. Verge, P.M. Richardson and R.J. Riopelle. Montreal General Hospital & McGill University, Montreal, Quebec, H3G 1A4 and Queen's University, Kingston, Ontario, K7L 3N6.

Approximately one half of the neurons in adult rat lumbar DRG have high-affinity receptors for NGF. To study changes in these neurons and receptors following peripheral nerve injury and subsequent infusion of NGF, radioautographs were prepared from cryostat sections of L5 DRG incubated with radioiodinated NGF. The right sciatic nerve was transected 30 days before sacrifice and in one half the animals, NGF was infused at 250ng/hr to the proximal nerve stump from the 21st to 30th days. By quantitative radioautography, the number of high-affinity sites on heavily labeled neurons was seen to fall by more than 80% after sciatic nerve transection, through loss in cell volume (50%) and receptor density (67%). For these neurons with high-affinity receptors, delayed infusion of NGF substantially reversed both receptor loss and atrophy. For neurons without high-affinity receptors, the mean volume was reduced only 25% by sciatic nerve transection and was not restored by NGF.

As part of the response to axonal injury and possibly because the cell body is deprived of NGF, fewer high-affinity receptors are displayed by sensory neurons. NGF can regulate its functional high-affinity receptor on adult mammalian neurons.

448.9

ACTIVATION OF CELL PROLIFERATION BY NERVE GROWTH FACTOR IN THE EMBRYONIC OTIC VESICLE AND COCHLEOVESTIBULAR GANGLION IN VITRO. J. Represa* and P. Bernd (SPON: J. Jakway). Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Brooklyn, NY 11203.

The effects of Nerve Growth Factor (NGF) on cell proliferation and survival in inner ear primordia (otic vesicle, OV; cochleovestibular ganglion, CVG) were studied. OV and CVG were from 72 hr quail embryos (stage 19-20); a period of active proliferation for these structures. They were incubated for 24 hr at 37°C in M-199 serumless medium containing ^{3}H-thymidine (4 μM; 10 μCi/ml). Duplicate cultures also contained either 10% fetal bovine serum, 50 ng/ml NGF, or serum plus NGF. Incubation with either NGF or serum resulted in a significant increase in the TCA precipitable incorporation of ^{3}H-thymidine in OV and CVG, as compared to those cultured in serumless medium alone (3x and 11x for OV; 4x and 6x for CVG). The combination of NGF and serum resulted in an additive response (12x for OV; 10x for CVG). Antibodies to NGF (1:100) blocked the effects of NGF, but not those of serum. In subsequent experiments, OV and CVG were growth-arrested by pre-incubation in serumless medium (24 hr), prior to reactivation with NGF and/or serum, for an additional 24 hr. Any cells dependent upon NGF or serum would probably not survive this 24 hr pre-incubation. Following reactivation, NGF elicited full proliferative effects as described above (blocked by anti-NGF), while serum only elicited a full response in OV. We conclude that NGF and serum cause specific increases in ^{3}H-thymidine incorporation in both OV and CVG. For NGF, this effect appears to occur by increasing cell proliferation, and not cell survival, since NGF is still able to elicit its full response in growth-arrested OV and CVG. Supported by grants from the NSF (BNS-8896101), March of Dimes (#1-1090), and Dysautonomia Foundation.

448.10

CHARACTERIZATION AND LOCALIZATION OF NERVE GROWTH FACTOR RECEPTORS IN THE EMBRYONIC OTIC VESICLE AND COCHLEOVESTIBULAR GANGLION. P. Bernd and J. Represa*. Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Brooklyn, NY 11203.

Recent findings have shown that Nerve Growth Factor (NGF) activates cell proliferation in inner ear primordia (otic vesicle, OV; cochleovestibular ganglion, CVG; see accompanying abstract), suggesting that at least some cells in these structures have receptors for NGF. OV and CVG from 72 hr quail embryos (stage 19-20) were incubated in M-199 serumless medium containing iodinated NGF (^{125}I-NGF; 15 pM to 24 nM; 1 hr; 37°C), while controls also included an excess of nonradioactive NGF (5 μg/ml). Quantitative analysis revealed specific ^{125}I-NGF binding (nonspecific binding was 25% or less of total binding), however, binding was not completely saturable even at the highest ^{125}I-NGF concentration (24 nM). Scatchard analysis indicated the presence of a single NGF receptor subtype, with dissociation constants similar to the low affinity form described in the periphery (5.2 nM, OV; 7.8 nM, CVG). OV were microdissected to determine the distribution of ^{125}I-NGF binding. The medial portion of the OV was found to contain approximately 65% of the label. Light microscopic radioautographic analysis was used to localize NGF receptors. Preliminary results reveal that ^{125}I-NGF binding is not uniform throughout either the OV or CVG. NGF receptors were concentrated in the ventromedial portion of the OV, the area adjacent to the CVG. The endolymphatic duct and lateral wall of the OV exhibited very little binding. In CVG, grain density was not homogenously distributed and all cells did not appear to have receptors for NGF. The phenotypic identity of the cells bearing NGF receptors remains to be determined. Supported by grants from the NSF (BNS-8896101), March of Dimes (#1-1090), and Dysautonomia Foundation.

448.11

Morphologically Distinct Populations of Cholinergic Neurons from the Basal Forebrain Exhibit a Differential Response to NGF or TPA. Z-W. Hua*, B. F. Alderson and L.B. Hersh. Lab. Developmental Neurobiol., NICHD, NIH, Bethesda. MD 20892.; Dept. Biochem., Univ. Texas Hlth. Sci. Ctr., Dallas. TX 75235.

Evidence from both *in vivo* and *in vitro* experiments indicate that nerve growth factor (NGF) modulates the level of choline acetyltransferase activity in cholinergic neurons of the basal forebrain. Previous reports have shown that the activation of protein kinase C by phorbol 12-myristate 13-acetate (TPA) can also increase the level of CAT activity. We have examined the effect of NGF and/or TPA on the number of CAT immunopositive cells in primary cell cultures from the basal forebrain region, in which three morphologically distinct cholinergic cell types; stellate, pyramidal, and bipolar, are evident (Nakajama et.al., PNAS, 82:6325, 1985).

Dissociated cell cultures were prepared from the basal forebrain region of 1-2 day old mouse pups and grown for 7 days following a single addition of NGF, 5-6 hours after plating. The maximal effective dose of NGF was found to be 200 ng/ml which produced a 1.9-fold increase in the total number of CAT positive cells/well and increased the number of stellate cells/well from 7±2 in the controls to 23±2. The addition of 100 ng/ml of NGF increased the number of pyramidal cells from 14±2 to 26±2 cells/well. However, no change in the number of bipolar cells was noted at any concentration of NGF tested. TPA,10.0 ng/ml, increased the total number of CAT positive cells from 27±5 to 42±4 cells/well. TPA increased the number of stellate cells from 4±2 to 12±2 and the number of pyramidal cells was increased from 11±2 to 20±3. The number of immunopositive bipolar cells was not changed by the TPA treatment. The study of the potential interaction of these two ligands is now underway.

448.12

AUTOCRINE DIFFERENTIATION OF RAT PHEOCHROMOCYTOMA PC12 CELLS USING A RETROVIRAL NGF VECTOR. M.P. Short*, M.B.Rosenberg, D. Ezzedine*, F.H. Gage, T. Friedmann* and X.O.Breakefield. E.K. Shriver Ctr., Waltham, MA 02254 and UCSD, La Jolla, CA 92093.

Rat pheochromocytoma PC12 cells have been genetically modified by use of replication-defective retroviral vectors containing, either the bacterial gene for beta-galactosidase (B-Gal) or the cDNA for mouse beta-NGF, and the bacterial gene for neomycin resistance.

Using the B-gal vector, clonal lines of PC12 cells were obtained in which almost 100% of cells stably expressed this histochemical marker. Infection of these B-gal-PC12 cells or the original PC12 cells with the NGF vector resulted in extensive neurite formation, which occurred within hours after infection and was maintained for weeks in culture. The percentage of cells expressing neurite outgrowth was comparable to that of PC12 cells treated with exogenous NGF. Neurite formation could be blocked by antibody to NGF. The amount of NGF in cells and that released into the medium was assessed by a two site radioimmunoassay and by a bioassay using "naive" PC12 cells.

Genetically modified PC12 cells are being injected stereotactically into the CNS of newborn and adult rats; their survival and effects on the brain are being assessed histologically.

448.13

THE EFFECTS OF PERTUSSIS TOXIN ON NERVE GROWTH FACTOR-STIMULATED RESPONSES IN PC12 CELLS. M.L. Contreras, K. Fujita,* P. Lelkes,* J. Shiloach, G. Guroff and P. Lazarovici. Section on Growth Factors, NICHD, and Laboratory of Cell Biology and Genetics, and Biotechnology Unit, NIDDK, NIH, Bethesda, MD 20892.

The specific events in the transmembrane signalling initiated by nerve growth factor (NGF) cells have not been identified. To test the possible involvement of a guanine nucleotide-binding protein, the effects of pertussis toxin on nerve growth factor-treated PC12 cells were examined. Incubation of PC12 membranes with pertussis toxin caused the ribosylation of a protein resembling the α-subunit of the guanine nucleotide regulatory protein, Ni. Incubation of PC12 cells with 1 μg/ml of pertussis toxin for 2 hours at 4°C inhibited both the NGF-induced increase in the intracellular concentration of free calcium and the NGF-stimulated hydrolysis of phosphoinositides. The inhibition of phosphoinositide hydrolysis was observed at concentrations of pertussis as low as 0.3 μg/ml, and was maximal at 1.8 μg/ml. When PC12 cells were treated with 1 μg/ml of pertussis toxin for two days, with fresh toxin added every 24 hours, NGF-induced neurite outgrowth was inhibited. Again, this inhibitory effect was observed at 0.3 μg/ml. The inhibitory effects were readily reversible; washing the cells to remove the toxin and readdition of NGF resulted in the production of neurites comparable to those in cells treated only with NGF. These results suggest that a pertussis-sensitive site, possibly a guanine nucleotide-binding protein, is involved in NGF receptor-linked signalling.

448.14

NGF, A DIFFERENTIATING AGENT, AND EGF, A MITOGEN, INCREASE THE ACTIVITIES OF DIFFERENT S6 KINASES IN PC12 CELLS. T. Mutoh,* B.B. Rudkin,* S. Koizumi* and G. Guroff. Section on Growth Factors, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892.

Soluble extracts from PC12 cells, prepared by the alkaline lysis method reported previously (Matsuda and Guroff, J. Biol. Chem., 262:2832, 1987), contain two S6 kinases, separable by heparin-Sepharose chromatography. As also shown in the previous study, the activity of the first of these peaks is increased in extracts from cells treated with nerve growth factor. We now report that it is the activity of the second of these peaks that is increased in cells treated with epidermal growth factor. In the presence of 80 mM β-glycerolphosphate and 20 mM EGTA, the NGF-responsive peak virtually disappeared; the EGF-responsive peak was resistant to this treatment. The EGF-responsive S6 kinase was not inhibited by the specific inhibitors of protein kinase A, H-7 and W-7. The characteristics of this EGF-responsive peak suggest that it is not protein kinase A, protein kinase C, or calcium/calmodulin-dependent kinase. It seems likely that the EGF-dependent kinase of PC12 cells is similar to the mitogen-responsive S6 kinase found in fibroblasts and other cells. The fact that NGF and EGF stimulate different S6 kinases in these cells indicates that these two peptides initiate different chains of intracellular mediators. These data open the possibility that agents inducing differentiation and those inducing proliferation produce different patterns of S6 phosphorylation, and that these, in turn, cause definable differences in S6 function.

448.15

EFFECTS OF NERVE GROWTH FACTOR ON CYTOSKELETAL GENE EXPRESSION IN AXOTOMIZED ADULT RAT DORSAL ROOT GANGLION (DRG) NEURONS. M.M. Oblinger and J.W. Wong* Dept. Cell Biology and Anatomy, Chicago Medical School, N. Chicago, IL 60064

While adult DRG neurons do not appear to be dependent on NGF for survival, NGF is thought to be important for the maintenance of normal metabolic and morphological homeostasis in these cells. In fact, the loss of trophic support from factors such as NGF as a result of axotomy has long been speculated to be involved in the injury response of sensory neurons. A very robust component of the axotomy response in DRG neurons is the cytoskeletal response. Recent studies by this and other laboratories have shown that peripheral axotomy of DRG neurons results in a downregulation of neurofilament (NF) proteins and mRNA levels and an upregulation of tubulin synthesis and mRNA levels. In the present study, we asked whether the administration of NGF to transected sciatic nerve would alter the cytoskeletal gene response in axotomized DRG neurons. To examine this question, the sciatic nerves of adult male rats were unilaterally transected at the midthigh level and placed into a silicone chamber which was connected to an implanted osmotic pump (Alzet). The transected sciatic nerves were continually infused (0.5 µl/hr) for 12 days with either NGF (0.5 mg/ml) or sterile saline. At 12 days, the axotomized and the contralateral L4 and L5 DRGs were harvested. The L4 DRGs were labeled for 1 hr *in vitro* with ^{35}S-methionine and the newly synthesized proteins analyzed by quantitative 2D gel electrophoresis/ fluorography. The L5 DRGs were embedded in paraffin, sectioned at 10 µm and hybridized with ^{35}S-labeled cDNA probes to the mRNAs of NF68 (provided by Dr. N. Cowan, NYU) and beta tubulin (provided by Dr. S. Farmer, Boston U). The quantitative results of both protein synthesis and *in situ* hybridization experiments indicated that NFG treatment did NOT alter the axotomy response with respect to the major cytoskeletal proteins. DRGs treated with NGF for 12 days after axotomy exhibited a substantial reduction in NF synthesis and NF68 mRNA levels and a substantial increase in tubulin synthesis and beta tubulin mRNA levels; the level of change in all measured parameters was not significantly different from that observed in saline treated axotomized controls. We conclude that this paradigm of administering NFG to axotomized DRG neurons does not alter the cytoskeletal component of the axotomy response.

448.16

DETECTION OF A PROTEIN KINASE ACTIVATED BY NERVE GROWTH FACTOR IN A HUMAN EWING'S OSTEOSARCOMA CELL LINE. C. **Volonté * and L.A. Greene** *Department of Pathology, Columbia University P&S, New York, N.Y. 10032.*

It has been previously found that several human Ewing's osteosarcoma cell lines possess NGF receptors (Thomson et al. [1988] Exp. Cell Res., 174: 533). One of these, the IARC-EW 1 line, responds to NGF by rapid induction of the c-*fos* proto-oncogene (Thomson et al., submitted). The present studies reveal that although this line fails to show certain responses to NGF seen in PC12 cell cultures, such as alteration of proliferation, change in morphology, survival in serum-free medium, altered glycoprotein synthesis and increased ornithine decarboxylase activity, it does share with PC12 cells the NGF-promoted activation of a serine protein kinase (PK) activity (designated PKN; Rowland et al. [1987] J. Biol. Chem., 262:7504). Partial purification of PKN from IARC-EW 1 cells by FPLC on a Mono-S column reveals at least a 10-fold activation after 10 min of NGF treatment. These findings suggest that the IARC-EW 1 line will be useful for probing the initial steps of the NGF mechanism of action. To facilitate the quantitative assessment of PKN activity (as well as the activity of other PKs) we have developed a new nitrocellulose slot-filtration assay. This assay, coupled with the rapidity and magnitude of PKN activation by NGF, may be convenient for detection of NGF responsiveness, especially in cells lacking macroscopic responses to the factor.

448.17

BEHAVIORAL AND BIOCHEMICAL EFFECTS OF NERVE GROWTH FACTOR IN RATS WITH 6-HYDROXYDOPAMINE INDUCED NIGROSTRIATAL DENERVATION. G. Pezzoli*, S. Fahn, A. Dwork*, J. de Yebenes*, V. Jackson-Lewis, J.L. Cadet. Dept. of Neurology, Columbia University, New York, New York 10032

The mechanisms by which grafts of catecholamine secreting cells alleviate the signs of experimental parkinsonism are still elusive. We undertook this study to evaluate the possibility that other factors, in addition to intracerebral transplantation of adrenal medulla, might play a role in these improvements. Aged Male Sprague-Dawley rats received 6-OHDA lesions of the substantia nigra. After several weeks during which they were evaluated for apomorphine-induced rotation, the rats were transplanted with adipose tissue and received intracerebral infusion of saline or nerve growth factor (NGF). One group received only NGF for a month period.

Behavioral studies revealed significant reduction in Apo-induced circling in the group treated with NGF + adipose tissue but not in the other groups. 6-OHDA caused significant decreases in DA, DOPAC, 5-HT, 5-HIAA in the ipsilateral striata. Adipose tissue transplantation exacerbated these changes in 5-HT and 5-HIAA. NGF significant attenuated the decreases in the 5-HT system without affecting the dopamine system. These results suggest that behavioral improvements seen after adrenal transplantation may be related to other factors in addition to the secretion of dopamine in the denervated nigrostriatal pathway.

448.18

IMMUNOREACTIVITY TO CHARACTERIZED GROWTH FACTORS IN RAT INTRASTRIATAL CELL SUSPENSION TRANSPLANTS. S.E.Loughlin, C.M. Annis* and J.H. Fallon. Dept. of Anatomy and Neurobiology, Univ. of California Irvine, Irvine, CA 92717

These studies were designed to determine whether characterized growth factors are present in the region of intrastriatal cell suspension transplants. Midbrains were removed from Sprague-Dawley rat embryos of 12 to 15 days of age. A suspension was prepared by incubation with trypsin , subsequent washing with trypsin inhibitor, DNA-ase, and MgSO4, and mechanical trituration. Suspension was injected into the caudate-putamen (CP) of adult rats which had previously received 6-hydroxy-dopamine lesions. Control animals received injections of vehicle only. Following varied survival times, animals were sacrificed by intra-aortic perfusion with fixative and brains were processed by the biotin-avidin-peroxidase immunocytochemical method. Antisera raised against tyrosine hydroxylase (TH), transforming growth factor alpha (TGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF) and glial fibrillary acidic protein (GFAP) were utilized. Dense TH-immunoreactivity (IR) was observed in cells and processes within the transplant, indicating that dopamine containing neurons survived and extended processes. No FGF-IR was observed in the region of transplants. In some animals, an increase in EGF-IR was observed around successful transplants. Densely staining profiles which appeared to be astrocytes (by comparison with GFAP-IR) were immunoreactive for TGF within and around sucessful transplants. When 3 well-characterized antisera directed at defined carboxy-terminal sequences of the TGF precursor were utilized, immunoreactivity in transplants was also observed. Injections of vehicle only produced a similar, but transient, effect. These data are consistent with the hypothesis that TGF may play a role in the functional improvement observed following fetal midbrain suspension transplants.

NEURONAL DEATH II

449.1

AN APPARENT ABSENCE OF CELL DEATH AMONG SPINAL CORD INTERNEURONS IN THE CHICK EMBRYO. S.E. McKay* and R.W. Oppenheim (SPON: B.T. Troost). Dept. of Anatomy, Wake Forest Univ., Winston-Salem, NC 27103.

Although naturally occurring neuronal death has been observed in a wide variety of neurons and species, there are reports in the literature indicating that some populations of neurons exhibit little, if any, loss during development. Because casual observations have suggested that degenerating interneurons are rarely, if ever, seen in the developing chick spinal cord we have undertaken a more systematic investigation of this problem. To determine whether spinal cord interneurons degenerate during development, we have examined every fifth section of the lumbar spinal cord on embryonic days (E) 6,8,10,12 and 15. This represents the time span when synaptogenesis occurs, and thus when cell death might be expected to occur. All regions of the spinal cord intermediate zone (gray matter) were searched for dying interneurons; however, motor and sympathetic preganglionic neurons were excluded from our analysis because previous studies have already documented cell death in these populations. Four-five embryos were examined at each age. Using previously established criteria we have observed few, if any, convincing examples of degenerating interneurons at any of the ages examined. We are presently carrying out a quantitative analysis of neuronal numbers at the same ages to more directly confirm the absence of interneuronal cell death. Experiments are also underway to determine whether loss of targets or afferents results in the death of spinal cord interneurons. Understanding why some populations do not undergo naturally occurring neuronal death may help to reveal the biological role of this process. This work was supported by grant NS 20402.

449.2

MECHANISM OF ACTION OF CONDITIONING NEURONAL LESIONS. G.R. Jackson*, K. Werrbach-Perez*, C. Beck* and J.R. Perez-Polo. Dept. of Human Biol. Chem. & Gen., Univ. of Texas Med. Br., Galveston, TX 77550.

Nerve growth factor (NGF) regulates neuronal cell death during development. The PC12 line is a useful model of an NGF responsive neuron. NGF provides protection to PC12 from hydrogen peroxide, H2O2, a well known hydroxyl radical generator. Exogenous catalase also protects and NGF does induce catalase in these cells that, like most neuronal lines, have low endogenous catalase levels. NGF protection is abolished by a catalase inhibitor. Here we report on a conditioning lesion paradigm that uses a sublethal peroxidative insult to stimulate protection to subsequent peroxidative exposure. Both NGF and the conditioning lesion itself independently confer cytoprotection from H2O2; together their effect is synergistic. When treated with NGF, conditioned cells display an acceleration of neurite outgrowth compared to unconditioned cells. This is consistent with results obtained in vivo and with the hypothesis that H2O2 can result in a xenobiotic response and that NGF regulates cell death via effects on oxidant-antioxidant balance. Supported by NIH grant NS-18708.

449.3

THE EFFECTS OF ION CHANNEL MODULATORS ON HYPOXIA-INDUCED CALCIUM ACCUMULATION AND INJURY IN CORTICAL NEURONAL CULTURES. M.L. Weber, A.W. Probert, P.A. Boxer, and F.W. Marcoux. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

Hypoxic neuronal injury in culture can be ameliorated by pharmacologic blockade at the NMDA gated ion channel. It has been proposed that inhibition of neuronal calcium accumulation accounts for this neuroprotective effect. We have evaluated the effects of calcium and sodium channel modulators on hypoxia-induced neuronal calcium accumulation and injury in culture. Two week old rat cortical neuronal cultures were exposed to hypoxia (0% O_2, 95% N_2, and 5% CO_2) at 37°C for 4 hrs in the presence or absence of ion channel modulators. After the hypoxic exposure neuronal $^{45}Ca^{++}$ uptake was measured. Sister cultures were returned to a normoxic environment (95% air, 5% CO_2) at 37°C for an additional 20 hrs and were examined for morphological injury after immunocytochemical staining with HRP linked neuron-specific enolase antibody. The percentage of injured cells was calculated by comparing photographs of identical microscopic fields taken before and after hypoxic exposure. Phenytoin, tetrodotoxin, verapamil, and diltiazem were found to block Ca^{++} accumulation yet they did not prevent neuronal injury. The inorganic cations, Mg^{++} and Zn^{++}, which block both voltage dependent and NMDA gated ion channels, inhibited Ca^{++} influx and protected the cells from morphologically assessed injury.

449.4

SURVIVAL OF CORTICOSPINAL NEURONS AFTER AXOTOMY IS TEMPORALLY CORRELATED WITH GROWTH OF THEIR AXONS INTO SPINAL CORD TARGETS. M. Merline* and K. Kalil. Dept. of Anatomy and Neurosciences Training Program, University of Wisconsin, Madison, WI 53706.

A previous report (Ramirez, L.F. and Kalil, K., J. Comp. Neurol., 237:506-518, 1985) describing effects of axotomy on pyramidal tract neurons showed that lesions in young hamsters did not produce cell death as shown with Nissl staining in layer 5 of the sensorimotor cortex. A subsequent report (Tolbert, D.L., and Der, T., J. Comp. Neurol., 260:299-312, 1987) showed complete degeneration of fluorescently labeled corticospinal neurons after axotomy in newborn kittens.

To resolve these discrepancies, we retrogradely labeled hamster corticospinal neurons with bilateral injections of rhodamine beads into different levels of the spinal cord, and 24-48 hours later made unilateral pyramidal tract lesions. Labeled neurons in the contralateral cortex served as controls. Neurons projecting to the lumbar cord completely degenerated if the pyramidal tract was cut before 10 days postnatal. Lesions at 14 days or older resulted in cell survival, although they were somewhat shrunken. In contrast, neurons projecting to the cervical spinal cord survived lesions of the pyramidal tract at 9 days of age, but lesions at 7 and 5 days produced progressively greater but by no means complete cell death. Short survival times used in some of the experiments showed retrograde cell changes as early as 24 hours after axotomy.

These results show that the age at which the lesion occurs determines the ability of corticospinal neurons to survive axotomy. Since corticospinal axons begin to innervate the cervical cord at 6 days and the lumbar cord at 10 days, the ability of corticospinal neurons to survive lesions of the pyramidal tract is temporally correlated with target innervation. (Supported by NIH Grant NS-14428)

449.5

THYROXINE-POTENTIATED LATERAL MOTOR COLUMN NEURON NUMBER IN LIMB AMPUTATED TADPOLES. J.F. Goldberg* and E.D. Pollack, Inst. Study Develop. Disabil. and Dept. Biol. Sci., Univ. Illinois at Chicago, Chicago, IL. 60608

The lateral motor column (LMC) neuron population of the developing frog is under the influence of both limb and thyroid hormone. Although neuron loss is temporarily retarded following limb amputation at the onset of dramatic LMC neuron loss, by the end of the larval period a substantial reduction in neuron number occurs. Since thyroid hormone influences spinal ventricular cell proliferation, we asked how limb amputation and thyroid hormone might interact in determining the LMC neuron number outcome in Rana pipiens tadpoles. Treatment with thyroxine by immersion resulted in an LMC with somewhat more neurons than controls. When hindlimb amputation is accompanied by continual thyroxine treatment, the LMC of the amputated side exhibits extraordinary numbers of neurons at the time of metamorphic climax. In fact, the neuron counts often exceeded those present at the time of amputation, when the maximum number is normally present. This suggested that neuron loss was being inhibited as expected, though perhaps for an extended time, while the rate of ongoing proliferation as determined by ventricular mitotic counts was accelerated by thyroxine, as was overall larval development. ^{3}H-thymidine autoradiographic analysis should confirm if the LMC neuron increase, in part, is a result of the thyroxine-enhanced mitotic activity.

449.6

MORPHOMETRIC ANALYSIS OF SCIATIC NERVE MOTOR NEURONS 20 WEEKS AFTER T-9 SPINAL CORD TRANSECTION. E.R. Feringa, R.L. McBride and J.K. Williams*. V. A. Medical Center and Medical College of Georgia, Augusta, GA 30910.

Functional recovery after spinal cord injury is dependent on the condition of neurons deprived of input by the trauma. We previously reported no loss of sciatic motor neurons 10 weeks after T-9 spinal cord transection and now we extend our observations to 20 weeks after transection.

We transected the spinal cord at T-9 in 7 anesthetized seven-week old female rats. Ten weeks later, the right sciatic nerve of these rats and 11 controls was severed above the popliteal fossa and the proximal cut end soaked for one hour in a 2% solution of the retrogradely transported fluorescent dye Fluoro-Gold. The rats were perfused 4 days later. Labeled neurons were counted on every fifth 30 µm section and measured on two sections from each rat.

In contrast with some earlier reports, we found that, at least as long as 20 weeks after T-9 transection, there is no significant change in sciatic motor neurons in number or size. In addition, the transected neurons retain their ability to retrogradely transport Fluoro-Gold. Supported by the V. A. Medical Research Service.

449.7

CORTICOSPINAL AND RED NUCLEUS NEURONS TRANSPORT FLUORO-GOLD 20 WEEKS AFTER T-9 TRANSECTION. R.L. McBride, E.R. Feringa, M.K. Garver* and J.K. Williams, Jr.* V. A. Medical Center and Medical College of Georgia, Augusta, GA 30910.

Although delayed death of corticospinal and rubrospinal neurons after T-9 spinal cord transection in adult rats was indicated by reduced anterograde (proline) and retrograde (HRP) labeling, the numbers of corticospinal axons at C-1 and T-1 did not decrease. To further investigate the status of neurons axotomized far from the somata, the spinal cords of 15 anesthetized seven-week-old female rats were completely transected at T-9. Ten weeks (7 transected, 8 controls) or 20 weeks (8 transected, 9 controls) later, a cotton pellet soaked in a 2% solution of the retrogradely transported fluorescent dye Fluoro-Gold was inserted into a new transection at T-1. Four days later the rats were perfused. The mean number and size of labeled corticospinal and number of rubrospinal neurons in transected rats was not different from controls at either 10 or 20 weeks. The mean size of labeled red nucleus neurons in transected rats was decreased compared with controls in both 10 and 20 week groups ($p<.001$). We conclude that some uptake/transport mechanisms remain intact following axotomy. Supported by the V.A. Medical Research Service.

449.8

TRANSNEURONAL DEGENERATION INVOLVES ACTIVE DESTRUCTION OF RIBOSOMES. E.W Rubel, P.M. Falk* and O. Steward (SPON: W.R. Lippe). Hearing Development Labs., Univ. of Washington, Seattle, WA 98195; Neurosci. Dept., Univ. of Virginia, Charlottesville, VA 22908.

We have shown that neurons in the avian n. magnocellularis (NM) which are destined to undergo transneuronal cell death due to interruption of afferent activity can be identified autoradiographically; cessation of protein synthesis occurs within 6 hours of eliminating afferent activity (Steward, O. & E.W Rubel, J. Comp. Neurol. 231: 385, 1985). In this study we examined ultrastructural cytoplasmic changes in these neurons. Chicks, 10-15 days after hatching, were subjected to unilateral cochlea removal. Six hours later they were briefly anesthetized and given a single intracardiac injection of 0.5 mci tritiated leucine. After 1/2 hr for incorporation, the brain stems were fixed and vibratome sectioned. Sections through the middle of NM were osmicated and embedded in plastic. Adjacent thick and thin sections were taken. Thick sections were processed for emulsion autoradiography. Grain counts over all NM neurons ipsi- and contralateral to the cochlea removal were determined. Electron micrographs of the cytoplasm of each neuron were examined, scored "blind" and related to the grain density for that neuron. We observed complete destruction of ribosomes associated with the endoplasmic reticulum and could find no cytoplasmic ribosomes in unlabeled neurons. These effects were striking and never observed in labeled cells from the experimental side or in cells from the normal side of the brain. The results suggest that afferent-regulated transneuronal cell death is an active process involving activation of a "suicide factor" through gene expression or enzyme disinhibition. (Supported by PHS grants NS24518 & NS12333)

449.9

CELL DEATH IN THE INFERIOR OLIVE OF STAGGERER MICE IS AN INDIRECT EFFECT OF GENE ACTION: STUDIES OF STAGGERER CHIMERAS AND +/*sg* MICE.

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In homozygous staggerer (*sg/sg*) mice, cerebellar Purkinje cells are reduced in number by 75% due to an intrinsic effect of the mutation on Purkinje cell development. In addition, 60% of the normal number of inferior olivary neurons are lost during postnatal development. To determine whether the olivary cell death is cell-autonomous (or caused by extrinsic factors such as the loss of most of the postsynaptic Purkinje cell target) chimeras were constructed from one *sg/sg, Gus^b/Gus^b* (high ß-glucuronidase activity) embryo and one *+^{sg}/+^{sg}, Gus^h/Gus^h* (low ß-glucuronidase activity) embryo. Three adult staggerer chimeras were identified. Despite the presence of both genotypes in the olive, cell counts revealed more *sg/sg* genotype neurons present in the chimeras than in a homozygous mutant. Since *sg/sg* olive neurons can be "rescued" in the chimera, their death must be an indirect consequence of *sg* gene action. Unexpectedly, heterozygote mice (+/*sg*), while behaviorally normal, lose 30% of their olivary neurons by 6 months of age. We have performed Purkinje cell counts in 12 month old +/*sg* animals and find a 30% loss of these neurons as well. Combined with data from the chimeras, these results suggest that the pattern of staggerer gene action is the same in both the heterozygote and homozygote animals.

Supported by the NIH (NS-20591 and NS-18381), the March of Dimes Birth Defects Foundation, and a FYSSEN Foundation Fellowship to HS.

449.10

UNIDIRECTIONAL REGULATION OF CELL NUMBER FROM TARGET TO AFFERENT NEURONS DURING DEVELOPMENT.

S. Chen and D. E. Hillman. Dept. Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Interactions between target and afferent neurons (or peripheral systems) are believed to generate a relatively precise numerical ratio through transneuronal cell death. Two models of developmental dysgenesis induced by methylazoxymethanol (MAM) in rats were used to reduce the number of Purkinje cells as targets (a single injection on gestational day 14) and the number of granule cells as afferents (a single injection within 24 hours of birth). The number of granule and Purkinje cells was quantitated in the adult control and two experimental groups from area and volume of layers and cell densities.

The results revealed that prenatal MAM on the 14th day produced a deficit in the number of Purkinje cells. This was accompanied by a parallel reduction of granule cells so that their ratio remained the same. Contrarily, major deficits in the number of granule cells, generated by postnatal MAM, did not affect the number of Purkinje cells. We conclude that the number of Purkinje cells determines the maximum number of granule cells as the result of a unidirectional control of afferent cell number. Supported by USPHS NS-20349 & NS-13742 from NINCDS.

449.11

ALZ-50 AS A MARKER FOR NATURALLY OCCURRING NEURONAL DEATH IN THE NEOCORTICAL SUBPLATE OF THE RAT.

W. Al-Ghoul* and M.W. Miller (SPON: R. Nagele) Department of Anatomy, School of Osteopathic Medicine and Robert Wood Johnson Medical School, UMDNJ, Piscataway, NJ 08854.

Neuronal death is a widespread phenomenon that affects many neurons in the developing nervous system. We used an immunohistochemical reaction to identify dying neurons in the subplate of the developing rat neocortex. Rats 0, 2, 5, and 30 days after birth were perfused with paraformaldehyde. Cortical tissue was reacted with a monoclonal antibody directed against Alz-50; Alz-50 is a 68K protein isolated from the brains of Alzheimer patients (Wolozin et al., Science, 232, 648 (1986)). Alz-50-immunoreactivity was visualized with a biotin-avidin-peroxidase technique. In neonates, immunoreactive neurons with round cell bodies were in the upper subplate and fusiform Alz-50-positive cells were distributed in the lower subplate. By postnatal day (P) 5 immunoreactivity in the subplate had disappeared. The time of origin of subplate neurons was determined using [^{3}H]thymidine autoradiography. These neurons were born on gestational day (G) 12. Heavily-labeled neurons were still evident on P5, but by P30 they have disappeared. Using a method which combined Alz-50 immunohistochemistry with [^{3}H]thymidine autoradiography, it was determined that many Alz-50-positive neurons were generated on G12. Thus, Alz-50 is expressed transiently and early in the process of neuronal degeneration. Funded by DE 07734, AA 06916, and AA 07568.

449.12

POST-HYPOXIC TREATMENT WITH MK-801 REDUCES HYPOXIC-ISCHEMIC NEURONAL DAMAGE IN THE NEONATAL RAT.

H. Hattori*, P.H. Schwartz, A.M. Morin, and C.G. Wasterlain*. (SPON: R. Nishimura) Epilepsy and Neurology Res. Labs., VAMC, Sepulveda, CA 91343, Dept. of Neurology, UCLA Sch. of Medicine.

We have examined the ability of post-hypoxic treatment of MK-801, a potent N-methyl-D-aspartate (NMDA) antagonist, to protect against hypoxic-ischemic brain damage in the neonatal rat. Both carotid arteries of 7-day-old rats were ligated. After 4-6 h, pups were exposed to 8% O_2 for 1 h. One group (n=8) received 10 mg/kg MK-801 (i.p.) just after the hypoxic insult. A second (n=8) received saline. Histological examination of the brains 3 days later revealed significant protective effects of MK-801. The percentage of necrotic area was scored as 0, +1 (1-25%), +2 (26-50%), +3 (51-75%) and +4 (76-100%). Post-hypoxic MK-801 reduced the degree of necrosis from +3.5±0.2 to +1.1±0.4 ($p<0.05$) in the neocortex, and from +2.1±0.3 to +0.6±0.3, ($p<0.05$) in the basal ganglia. Protection was not selective and included neocortical astrocytes. The data suggest that MK-801 has the therapeutic potential in neonates even when given after the hypoxic-ischemic insult.

449.13

THE REACTION OF GRACILE PROJECTING PRIMARY SENSORY NEURONES FOLLOWING PERIPHERAL NERVE INJURY IN THE RAT.

J. K. E. Persson*, H. Aldskogius, J. Arvidsson* and A. Holmberg*. Dept. of Anatomy, Karolinska Inst., 104 01 Stockholm, Sweden.

The purpose of this study was to analyse qualitative and quantitative morphological changes in gracile projecting primary sensory neurones following sciatic nerve transection in the rat. The possible occurence of ultrastructural changes in the gracile nucleus was examined at post-operative (p.o.) survival times ranging from one day to 32 weeks. Degenerative changes were found in the ipsilateral gracile nucleus from three days up to 32 weeks after nerve transection. These changes in terminals, preterminal axons and glial cells differed markedly from previously described transganglionic changes in other systems. Transganglionic labelling from the injured sciatic nerve with horseradish peroxidase (HRP) at the ultrastructural level showed that at least some of the altered structures were indeed the central processes of primary sensory neurones whose peripheral branches were located in the sciatic nerve. The number of dorsal root ganglion neurones in dorsal root ganglia L4 to L6 projecting to the gracile nucleus was counted 20 weeks after nerve transection. This quantitative analysis showed an ipsilateral decrease of about 30% in the number of cell bodies compared with the contralateral unoperated side. The number of axons in the gracile funiculus at C3 spinal cord level was calculated 20 weeks after ipsilateral sciatic nerve transection. The counting was restricted to an area containing axons which had been labelled with HRP from the sciatic nerve. This quantification showed a reduction of about 25% in the number of axons compared with age matched controls. In material prepared with the Marchi method to visualize degenerating myelin, a slight increase in the number of Marchi-positive structures was noted in the gracile funiculus ipsilateral to nerve transection compared with the contralateral unoperated side and age matched controls.

These findings indicate: i) that sciatic nerve transection results in prominent alterations in central terminals and axons of the injured gracile projecting ganglion cells and ii) that the occurence of these alterations are accompanied with ganglion cell death.

449.14

AGENTS WHICH PREVENT THE DEATH OF NEURONS CAUSED BY NERVE GROWTH FACTOR (NGF) DEPRIVATION

Beth K. Levy*, D.P. Martin, and E.M. Johnson, Jr. (SPON: R.E. Schmidt) Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110

Elevated cAMP prevents neuronal death caused by NGF deprivation. Superior cervical ganglia were dissected from embryonic day-21 rats and dissociated onto collagen-coated tissue culture dishes. After one week in the presence of NGF, cultures were acutely deprived of trophic support by adding anti-NGF antiserum. Neurons treated in this manner were completely dead 48 hours after NGF deprivation. Consistent with Rydel and Greene (PNAS 85:1257-1261), chlorophenylthio-cAMP (CPT-cAMP), forskolin, cholera toxin, and isobutylmethylxanthine (IBMX) all prevented neuronal death. A concentration of IBMX too low to save by itself markedly lowered the EC_{50} of CPT-cAMP and forskolin. The ability of raised levels of cAMP to prevent neuronal death may represent a pharmacologic, rather than physiologic, effect because NGF deprivation did not decrease intracellular cAMP.

Retinoic acid prevented the death of NGF-deprived neurons as did elevated concentrations of potassium in the medium. Neither of these treatments raised intracellular levels of cAMP. We have previously shown that the death of NGF-deprived neurons requires RNA and protein synthesis. CPT-cAMP, retinoic acid, or elevated potassium did not decrease protein synthesis sufficiently to explain their saving effect, even after several days.

A variety of agents failed to alter the death of NGF deprived neurons. These included phorbol esters, polyamines, protease inhibitors, kinase inhibitors, drugs which alter the cytoskeleton, and others.

449.15

NEURONAL DEATH CAUSED BY NERVE GROWTH FACTOR (NGF) DEPRIVATION RESULTS FROM A CASCADE OF NEW RNA AND PROTEIN SYNTHESIS D.P. Martin and E.M. Johnson, Jr. Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110

We have further characterized an in vitro model of trophic factor deprivation in which we have previously shown that neuronal death requires RNA and protein synthesis. Comparison of the dose response curves of cycloheximide for inhibition of protein synthesis and neuronal saving indicated that protein synthesis must be inhibited at least 80% to prevent neuronal death.

Inhibitors of RNA or protein synthesis prevented neuronal death only if added within about 18 hours of NGF deprivation. This time is coincident with the first observable morphologic changes associated with neuronal death, which once initiated apparently progress independent of RNA or protein synthesis. The commitment point was also determined for other agents which prevent neuronal death (NGF, CPT-cAMP, high potassium concentrations, and retinoic acid).

Neurons were deprived of NGF for 24 hours in the presence of cycloheximide, whereupon the protein synthesis block was removed without replacing NGF. These cells did not die abruptly, but rather synthesized protein for an additional 18-24 hours before beginning to die. Hence, RNA which codes for the proximate killer proteins does not accumulate during the 18 hour lag between NGF deprivation and commitment.

We believe that these data are most consistent with the hypothesis that NGF deprivation unleashes a cascade of sequential RNA and protein synthesis ending in the production of new proteins which kill the cell.

449.16

NEURONAL DEATH CAUSED BY CYTOSINE ARABINOSIDE (ARA-C) RESEMBLES DEATH CAUSED BY NERVE GROWTH FACTOR (NGF) DEPRIVATION T.L. Wallace, D.P. Martin, and E.M. Johnson Jr. Dept. of Pharmacology, Washington Univ. School of Medicine, St. Louis, MO 63110

We report here that Ara-C causes the death of rat sympathetic neurons in a fashion resembling that caused by NGF deprivation. Superior cervical ganglia were dissected from embryonic day-21 rats and dissociated onto collagen-coated tissue culture plates. After a week in the presence of NGF they were exposed to Ara-C (10^{-4}M). No changes were observable until about three days, whereupon the neurites began to thin and fragment. Over the next 24 hours the cell bodies became condensed and phase dark, such that by five days after addition of Ara-C all neurons in the dish were completely destroyed. The morphological and temporal characteristics of cell death that began around three days after adding Ara-C were very similar to those observed starting 24 hours after NGF deprivation. Although Ara-C is an antimitotic drug, its action here does not involve DNA synthesis because these neurons are postmitotic and unharmed by any other antimitotic drug (Ara-A, Ara-T, and FUDR). As with neuronal death caused by NGF deprivation, the death caused by Ara-C was prevented entirely by inhibitors of protein and RNA synthesis, agents which elevate intracellular cAMP, and elevated concentrations of potassium in the culture medium. These data suggest that Ara-C causes an active death of neurons similar to that caused by trophic factor deprivation.

RETINA VI

450.1

RETINAL TOPOGRAPHY AND THE RETINOTECTAL PROJECTION PATTERN IN THE JUVENILE LEMON SHARK (NEGAPRION BREVIROSTRIS). R.E. Hueter. Mote Marine Laboratory, Sarasota, FL 34236.

Attention to the topography of shark retina has been hindered by the obsolete view that the elasmobranchs as a rule possess all-rod retinas. After Gruber et al. (Vision Res. 3:397, 1963) demonstrated conclusive evidence of cones in the lemon shark, at least 24 species from 10 families of elasmobranchs with duplex retinas have been described. In this study, unstained retinal wholemounts and Nomarski DIC optics were used to map the topographic organization of cones and ganglion cells in juvenile lemon shark retina. Retinal cell distribution is organized into a visual streak in register with the retinotectal streak, which is oriented within about 15° above and 15° below the horizontal meridian (Hueter, R.E., Soc. Neurosci. Abs. 12:496, 1986). Ganglion cell density ranges from a high of over 1500 cells/mm^2 inside the streak to less than 500 cells/mm^2 in the periphery. Cone density is approximately 6500 cones/mm^2 inside the streak and less than 500 cones/mm^2 in the extreme periphery. Average intercone separation is calculated to be 12.4 µm at peak cone density, which, with a posterior nodal distance of 9.398 mm in this eye, corresponds to a visual angle of 4.5'. This suggests that the visual system of the lemon shark may be specialized for certain discrimination tasks and calls for further research on spatial vision in sharks.

Supported by the Lerner-Gray Fund for Marine Research and a Sigma-Xi Grant-in-Aid.

450.2

TOPOGRAPHIC ANALYSIS OF THE GANGLION CELL LAYER AND OPTIC NERVE IN THE SANDLANCE, LIMNICHTHYES FASCIATUS (CREEIIDAE, PERCIFORMES). S.P. Collin* and H.B. Collin* (SPON: A. Hughes). Vision, Touch and Hearing Research Centre, Department of Physiology and Pharmacology, University of Queensland, St Lucia, Queensland 4067, Australia and School of Optometry, University of New South Wales, Kensington 2033, N. S. W. Australia.

The sandlance *Limnichthyes fasciatus* is a small teleost of the Indopacific which lives beneath the sand. Its miniscule eyes (1.04mm in diameter) are independently mobile, possess a unique cornea and a deep convexiclivate fovea on the visual axis. Examination of Nissl-stained cells within the ganglion cell layer reveal a perifoveal density of 13.0 X 10^4 cells per mm^2 counted in wholemount material compared to 15.0 X 10^4 cells per mm^2 counted in transverse sections. Peripheral densities drop to 4.5 X 10^4 cells per mm^2. To assess the proportion of cells without an axon, sections of the whole optic nerve were analysed under electron microscopy. Optic axon densities range from 2 X 10^6 axons per mm^2 in the caudal apex to over 16 X 10^6 axons per mm^2 within a specialized region of unmyelinated axons in the rostral apex. The unmyelinated axon population (26%) follows closely the topography of the total population of axons. A total of 104,452 axons were found in the optic nerve compared to 102,918 cells situated in five sublaminae within the retinal ganglion cell layer. A direct relationship is revealed between ganglion cell soma size and axon area. The corresponding topographic organization of the retina and optic nerve may reflect some functional retinotopicity.

450.3

NUMBER, DISTRIBUTION, AND RATIOS OF RODS AND CONES IN THE ADULT MACAQUE RETINA. K.C. Wikler, R.W. Williams, and P. Rakic. Section of Neuroanatomy, Yale University School of Medicine. 333 Cedar Street, New Haven, CT 06510

The photoreceptor matrix is a key determinant of visual acuity. However, the number and distribution of rod and cone photoreceptors has not yet been fully characterized in the primate retina. We have estimated total photoreceptor number, topography, and ratios in adult rhesus monkeys using differential interference contrast (DIC) optics and unstained and hydrated wholemount retinae (Curcio et al., 1987, Science, 236, 579-582).

There are 3.2 million cones (95% confidence interval (CI) ± 290,00) and 62 million rods (CI ± 500,000) in the adult monkey retina (n=7). The average density of cones in the rod-free foveola is 150,000 cells/mm^2 but values range widely from 88,000 to 213,000 cones/mm^2. Since these measures were made using identical counting procedures, the wide range of values in peak cone density may be due to difficulty in consistently visualizing the exact location of this spatially restricted rod-free zone.

The distribution of cones shows a prominent 10:1 gradient in density from central to peripheral retina. Outside of the foveola, rods are found also in higher density in central retina (5:1 center-periphery ratio). In contrast, 5 mm outside of the foveola, the ratio of rod to cone density is relatively uniform across the retinal surface. Ratios in this broad plateau region (90% of retinal area) range from 15-30:1 rods per cone. Although the density of cones per surface area decreases dramatically in the periphery, surprisingly, the number of cones per rods is stable across the retinal mosaic.

Supported by EY02593.

450.4

TOPOGRAPHIC ORGANIZATION OF THE GANGLION CELL LAYER IN THE RETINA OF THE ADULT BABOON (PAPIO ANUBIS) O. Fischer*, P. Wilson, and M. Kirby[1], University of California, Riverside, Riverside, CA 92521 and [1]Loma Linda University, Loma Linda, CA 92350.

The distribution of ganglion cells in the retina of the baboon, a terrestrial monkey, was determined for comparison with retinas from more arboreal primates. Retinas were immersion fixed, whole mounted and stained with cresyl violet. The retinas measured from 738 - 948mm^2 in area, and contained an estimated 1,220,000 - 1,570,000 (mean 1,430,000) ganglion cells, with a peak density of at least 18,000 cells/mm^2. Ganglion cell counts within the central 3-5% of the retinas may be underestimated because of the extreme density and thickness of the ganglion cell layer in this region.

The distribution of ganglion cells outside central retina forms a pronounced horizontal "streak." The ratios of the long to short axes of the 1000 cells/mm^2 and 2000 cells/mm^2 isodensity contours were 1.6:1 and 1.9:1 compared with ratios of 1.2:1 to 1.4:1 for comparable isodensity contours in retinae of more arboreal primate species (extrapolated from the data of: Stone, J. et al J. Comp. Neurol., 196:205, 1981; and Perry, V. H., et al. Vision Res., 25: 1795, 1985). This data is consistent with the hypothesis that the visual streak tends to be more pronounced in ground dwelling than in arboreal species (Hughes, A., Handbook of Sensory Physiology 7/5, 1977).

450.5

VISUAL ACUITY OF THE CAT: VARIATION WITH RETINAL ECCENTRICITY. *Kris Horn and Tatiana Pasternak.* University of Rochester Medical School and Center for Visual Science, University of Rochester, Rochester, NY 14627.

Anatomical and physiological studies of the cat retina provide estimates of the limits of spatial resolution at various retinal eccentricities. Estimates based on the density of ganglion cells, dendritic field diameters and receptive field center diameters suggest that X (beta) cells limit spatial resolution in the cat. Such predictions for areas outside of the central retina have never before been verified by psychophysical testing.

We developed procedures that enabled us to behaviorally measure visual function at any retinal eccentricity. We measured grating acuity in cats, whose eye position was monitored by means of a scleral search coil technique. During behavioral testing, the cat was placed in an apparatus equipped with a head restraining device and two adjacent response pedals. During each trial the cat was required to maintain fixation on a laser spot and respond to the presence or the absence of a grating by pressing the right or the left pedal. The cats readily adjusted to these training conditions and we were able to measure acuity along the horizontal and vertical meridia at eccentricities up to 12 deg in the nasal, temporal, superior and inferior retina. Acuity in area centralis reached about 4 c/deg and it declined by a factor of two at 4 deg and a factor of 3-4 at 12 deg eccentricity. The acuity was higher in the nasal than temporal retina. At all eccentricities, the behavioral acuity exceeded the resolution limit derived from Y(alpha)- cell properties, but was consistent with the values derived from the X (beta) cell properties. Supported by T32 MH18260, EY06175, EY01319.

450.6

VISUAL ACUITY ACROSS THE MACAQUE RETINA. W. H. Merigan, L. M. Katz, Dept. Ophthalmology, Univ. Roch. Med. Ctr. Rochester, N.Y. 14642.

The variation of macaque visual acuity with eccentricity was mapped behaviorally from the fovea to 30 deg eccentricity along nasal and temporal horizontal meridia. These results can be interpreted in terms of visual properties which change with eccentricity such as optical quality, cone density, ganglion cell density, and dendritic and receptive field dimensions.

Acuity was measured by the discrimination of vertical from horizontal sinusoidal gratings (17 cd/m2 mean luminance) to minimize the use of aliased information. Fixation locus was controlled behaviorally and monitored with scleral search coils. Acuity was determined at 0, 3, 6, 12, 20, and 30 deg eccentricity in an interleaved series, and stimulus size was approximately 10 cycles of a just resolved grating.

Visual acuity decreased more sharply across temporal than nasal retina, and this difference was slightly greater than that seen in humans. The rate of decline was consistent with cone density over the central 10 deg of the retina and with the density of P ganglion cells at greater eccentricities, although it has been shown that such densities cannot in themselves limit acuity.

Comparison of our acuity results with dendritic and receptive field dimensions suggest that spatial averaging may play some role in limiting visual acuity. Supported by grants NSF BNS- 8518858, ES01247, and EY01319.

450.7

SELECTIVE NEUROTOXIC DAMAGE IN THE SQUIRREL MONKEY TO THE PARVOCELLULAR RETINO-GENICULATE PATHWAY. J.J. Lynch III*, W.H. Merigan and T.A. Eskin, Environ. Hlth Sci Ctr and Depts. of Ophth. and Pathol., Univ Roch Med Ctr, Roch., NY 14642.

We have previously found, in acrylamide-exposed macaques, selective degeneration of those retinal ganglion cells that project to parvocellular layers of the lateral geniculate nucleus(dLGN)(Eskin and Merigan, 1986). While the basis of the selectivity is not yet known, this lesion provides a useful model for the study of the functional role of parallel visual pathways. We now report a similar selective lesion in the visual system of a New World monkey.

Four male squirrel monkeys (Saimiri sciureus) were given daily oral doses of 14 to 21 mg/kg (494 to 1680 mg/kg total dose) acrylamide monomer over periods of one month to several months and then allowed to recover for 3 to 4 months. Radial sections of the retina of these monkeys showed a 50 to 80% loss of ganglion cells. WGA-HRP transport from retina demonstrated a nearly complete loss of transport to all parvocellular layers of the dLGN, but largely normal transport to magnocellular layers.

Cytochrome oxidase activity was greatly decreased throughout the parvocellular layers while magnocellular layer activity was maintained. Cytochrome oxidase activity in the striate cortex was decreased in parvocellular recipient layer 4C beta, but showed little decrease in magnocellular recipent layer 4C alpha.

These results suggest that retinal ganglion cells of the parvocellular pathways of the squirrel monkey and macaque share some feature which makes them vulnerable to this chemical. The selective lesion of the parvocellular pathway reported here can be used in the squirrel monkey, as in the macaque, to examine the functional role of this pathway. Supported by EPA 812402 and ES01247.

450.8

MORPHOLOGY OF GANGLION CELL TYPES THAT PROJECT TO THE PARVOCELLULAR LAMINAE OF THE LATERAL GENICULATE NUCLEUS, PRETECTUM, AND SUPERIOR COLLICULUS OF PRIMATES. R.W. Rodieck and M.Watanabe Department of Ophthalmology, University of Washington, Seattle WA 98195

Macaque ganglion cells were retrogradely labeled following 1 µl injections of dextran-labeled fluorescent dyes to brain regions that received a direct retinal input. Following a one-week survival time the animal was sacrificed, the eyes hemisected, and the retina removed and placed in an *in vitro* chamber, where it was suprafused with Ames medium. The chamber was placed on the stage of an epifluorescence microscope and the labeled cells visualized using a water-immersion objective. The labeled cells were intracellularly injected with horseradish peroxidase, and the retinas were later reacted with DAB, and whole mounted.

The great majority of ganglion cells that project to the parvocellular laminae of the LGN are midget ganglion cells, as previously reported (Leventhal, Rodieck and Dreher, 1981). In addition, these laminae receive from ganglion cell types not previous described, including some with dendritic field diameters up to 860 µm (the largest primate ganglion cells we have yet observed). The pretectum is dominated by ganglion cells with large dendritic fields formed from dendritic processes that seldom branch. Most of the ganglion cells that project to the superior colliculus belong to one of three main groups, which we have tentatively termed thorn, maze, and sparse. Thorn cells have a thick dendritic field whose terminal processes ramify at one of two levels within the inner plexiform layer. Maze cells stratify within a narrow zone of the IPL. Sparse cells seldom branch, and have large dendritic-field diameters (~400 µm) that change little with retinal eccentricity.

Suppored by NIH grants EY-01730, EY-02923 and the E.K. Bishop Foundation.

450.9

FORM OF RABBIT RETINAL GANGLION CELLS PROJECTING TO LAYERS OF THE SUPERIOR COLLICULUS. J.E.G. Downing*, E. V. Famiglietti, B. Ferguson*, and T. Shaw*. Lions Sight Centre, University of Calgary, Calgary, AB, Canada T2N 4N1.

Studies were initiated to assess the organization of parallel pathways from identified retinal ganglion cells (GCs) to the superficial layers of the superior colliculus (SC) in rabbit. In cat, W-GCs provide crossed projections (Fukuda and Stone, '74) to the upper stratum griseum superficiale (SGS) (Berson,'87), while Y-GCs provide both crossed and uncrossed projections (Fukuda and Stone, '74) to the deeper portion of the SGS (Hoffman, '73; McIlwain and Lufkin,'76). In rabbit, superficial and deep SGS contain functionally different populations of neurons (Graham et al.,'81). Thus while most GCs have axonal branches in SC (Vaney et al.,'81), it is possible that different functional types of GC have different sublaminar and hence parallel, rather than strictly convergent projections. To address this possibility we have made localized deposits of HRP or rhodamine-latex beads in the superficial SC, either by pressure injection, or by a method of linear deposition (Bowling,'87), for retrograde labelling of GCs. Two days later retinas are removed and processed histologically (DAB-HRP), or placed in a perfusion chamber, and bead-labelled GCs stained with Lucifer yellow by iontophoresis. We will describe sublaminar differences in projections of GCs, identified by cell body size and dendritic branching. (Supported by the Alberta Heritage Fnd. for Med. Res. and the MRC of Canada.)

450.10

TRANSNEURONAL LABELING OF RETINAL CELLS AFTER INJECTION OF HERPES SIMPLEX VIRUS INTO OPTIC NUCLEI. R.B. Norgren, Jr.* and M.N. Lehman (SPON: G. Kokoris), Dept. of Anat. & Cell Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH 45267.

Herpes Simplex Virus, Type 1 (HSV-1) has generated great interest as a possible transneuronal tracer. We have examined the transport of HSV-1 in the optic system. 40-80 nl of HSV-1 (McIntyre strain) were injected into the hypothalamic suprachiasmatic nucleus (SCN) or superior colliculus (SC) of adult golden hamsters. After a survival period of 3 days animals were perfused with 4% paraformaldehyde, eyes and brains were removed and sectioned either on a freezing microtome or a cyrostat. HSV-1 infected cells were visualized with an antibody (Accurate) and an avidin-biotin-HRP procedure (Vectastain). Brain sections were examined to verify injection sites. In addition to damage created by the syringe, there was extensive necrosis in the vicinity of the injection as a result of viral infection. Neurons in nuclei afferent to the injected sites were heavily labeled. Examination of retinal sections revealed labeled ganglion cells in a distribution similar to that observed after injection of traditional retrograde tracers: after SCN injections, labeled ganglion cells were found dispersed throughout the retina. In contrast, the retinae of SC injected animals exhibited areas where many adjacent ganglion cells were labeled. After injections into either region, infected cells were also found in the inner nuclear layer (INL) adjacent to labeled ganglion cells; the proximity of labeled INL cells suggests that after retrograde transport of the virus to the ganglion cells, it consequently infects neurons in synaptic contact with the labeled ganglion cells. We are currently testing this hypothesis at the electron microscopic level. [Supported by NIH NS24292 to M.N.L.]

450.11

AFFERENT AND EFFERENT CONNECTIONS OF THE ISTHMO-OPTIC NUCLEUS IN PIGEON (*Columba livia*). Woodson, W.*, T. Shimizu, and H. J. Karten. Department of Neurosciences, M-008, School of Medicine, University of California, San Diego, La Jolla, CA 92093.

Previous studies (McGill et al.,'66 a,b) have shown that the isthmo-optic nucleus (ION) is the source of a projection (centrifugals) upon the retina, and receives a topographic projection from the optic tectum. The afferent and efferent connections of this nucleus were identified using Phaseolus vulgaris leucoagglutinin (PHA-L). Retrogradely labelled cells were found at the margin of layers 9-10 throughout the rostro-caudal extent of the optic tectum. The dendrites of these tectal neurons extend into the retino-recipient layers, 2-7. Efferents of ION terminate in the contralateral ION. Centrifugals to the retina arborize in layer lb and, more sparsely, in layer 5b of the inner plexiform layer (IPL). Many processes could be followed into the inner nuclear layer, forming pericellular nests and "palmate" endings around somata of medium-large sized amacrine cells. These results indicate that, in addition to direct input upon somata of amacrine and displaced ganglion cells, the centrifugals also terminate within sharply defined laminae of the IPL. Furthermore, the organization of the isthmo-optic efferents suggests that the optic tectum is able to exert bilateral control over discrete subpopulations of amacrine cells in the retina. Supported by Grants NEI-EY06890 to HJK and the Ford Foundation to WW.

450.12

AXON COLLATERALS AND/OR EFFERENT FIBERS IN THE MONKEY RETINA. C. Usai*, S. Bisti~*and S. Vallerga*.(SPON: European Brain & Behavior Society).Ist.Cib.e Biofisica,CNR, 16146 Genova and ~Ist. Neurofisiol.,CNR, 56100 Pisa, ITALY.

The presence in the monkey retina of long axons with many collaterals ending as knobbed branchlets at the surface of the inner nuclear layer (INL), has been reported by Perry et al.(*Neurosci*.12:1101,1984) after injection of HRP in the optic nerve. Those collaterals were tentatively ascribed to centrifugal fibers.

In reduced silver stained retinae of *Macaca fascicularis* we observed several fibers, branching repeatedly, often emerging from axon bundles, resembly the fibers reported in HRP material. In one instance we could trace the origin of collaterals to the axon of a small neuron with large dendritic field, located in the ganglion cell layer of the inferior nasal hemiretina. The collaterals travel for several mm. through the inner plexiform layer (IPL), keep preferentially orthogonal to axon bundles, reach on occasion the INL, and cover mostly the nasal region. Therefore both centrifugal fibers and ganglion cells originating association axon collaterals might be present in monkey retina.

450.13

INTRINSIC AXON COLLATERALS OF TURTLE RETINAL GANGLION CELLS. W. Gardiner* and D.M. Dacey (SPON: M. Koontz). Dept. of Biological Structure, The Univ. of Washington, Seattle, WA 98195

Intracellular injections of horseradish peroxidase (HRP) reveal a distinct ganglion cell type that gives rise to intrinsic axon collaterals terminating in the inner plexiform layer (IPL). Retinas from the pond turtle (*Pseudemys scripta elegans*) were dissected and placed, vitreal surface up, in a superfusion chamber mounted on the stage of an epifluorescent microscope. Application of the vital dye, acridine orange to these retinas produced a stable fluorescent staining of ganglion cell somata *in vitro*. Intracellular injections of lucifer yellow and rhodamine labelled HRP were made into the fluorescing cells under direct visual control.

The axon collateral bearing ganglion cells have large somata (~20 μm diam) and large dendritic fields (~700-1000 μm). Their dendritic tree is narrowly stratified at the outer border of the IPL (~40 μm from the soma). Dendrites are thick, wavy, moderately branched and extend radially from the soma without much overlap. The parent axon (~1-2 μm diam) arises from the soma and courses directly to the optic disc in the nerve fiber layer, issuing one or two thin axon collaterals near the soma. The primary collateral descends directly into the IPL, branching several times to form a distinct terminal arbor that is monostratified at the inner border of the IPL (~5 μm from the soma). The terminal arbor has a diameter that is approximately the same size as, and partially overlaps, the dendritic field. The axon collaterals bear distinct terminal *boutons* along their length.

Because the axon collaterals overlap the dendritic tree, they may provide recurrent feedback via amacrine or interplexiform cells. Alternatively, the difference in stratification between the dendritic tree, in the OFF-sublamina of the IPL, and the intrinsic axon collaterals, in the ON-sublamina of the IPL, suggest a synaptic pathway allowing OFF-center ganglion cells to directly influence the ON pathway.

450.14

CHARACTERIZATION OF SOMATOSTATIN-LIKE IMMUNOREACTIVE CELLS IN THE ADULT CAT RETINA. **C.A. White, D. Johnson[1], L.M. Chalupa and N.C. Brecha[1], Dept. of Psych., UCD, Davis, CA and Depts. of Med. & Anat., UCLA, L.A., CA[1]**

We have studied somatostatin-like immunoreactivity (SRIF-I) in the adult cat retina using a mouse monoclonal antibody (from Dr. A. Buchan) directed to $SRIF_{14}$. A quantitative analysis of one retina, representative of 8 others, was carried out. Two distinct groups of SRIF-I somata, both distributed preferentially in inferior retina, were found. The first group consisted of large cells with granular staining cytoplasm and poorly stained primary processes. There were 561 such cells, all in the ganglion cell layer (GCL). Their density ranged from 0-2 cells/mm^2 in superior retina to an average of 4 cells/mm^2 in inferior retina. Density peaked at 11 cells/ mm^2 in a region about 4 mm inferior to the area centralis. The average soma area of a sample from inferior nasal retina was 742 μm^2. An analysis of cell distribution in this region found the pattern to be non-random (chi square test, p<.001) with an average nearest neighbor distance of 347 μm.

The second cell type was characterized by darkly stained, small to medium somata with 2-4 primary processes. There were 1,782 such cells in the GCL and 153 in the inner nuclear layer (INL). The density of cells in the GCL averaged about 5 cells/mm^2 in inferior retina except at the retinal margins, where it increased to 43 cells/mm^2. There were very few cells in superior retina, except at the retinal margin, where density was high. The average soma size in inferior nasal retina was 237 μm^2 for cells in the GCL and 170 μm^2 for cells in the INL. An analysis of the distribution of cells in this region found the GCL cells to be distributed non-randomly (chi square test, p<.01) with an average nearest neighbor distance of 228 μm. (Supported by NINCDS T32 NSO7300, EY03991 and EY04067.)

450.15

AXON-BEARING AMACRINE CELLS OF THE PRIMATE RETINA. D.M. Dacey. Dept. of Biological Structure, Univ. of Washington, Seattle, WA 98195.

A new, and unprecedented type of retinal amacrine cell has been identified in the primate retina by intracellular injections of horseradish peroxidase (HRP). Freshly dissected macaque monkey retinas were placed in a tissue chamber mounted on the stage of a light microscope and continuously superfused with oxygenated Ames medium. Application of the vital dye, acridine orange, to these retinas produced a stable fluorescent staining *in vitro* of the somata of apparently all retinal cells in the inner nuclear layer and ganglion cell layer. Large cell bodies (~20 μm diam) were also consistently observed in the middle of the inner plexiform layer (IPL). Intracellular injections of HRP into these large cells under direct microscopic control showed that they comprise a single, morphologically distinct amacrine cell subpopulation. The dendritic tree of this cell type is moderately branched (~40-50 terminal dendrites) and broadly stratified, spanning the central ~50% of the IPL such that the soma is situated between the outermost and innermost dendritic branches. Dendritic field diameter increases from ~200 μm within 1.5 mm of the fovea to ~500 μm in the periphery. Injections of patches of the axon-bearing amacrines revealed a regular cell to cell spacing (~200-300 μm in the retinal periphery) suggesting the formation of dendritic territories and a constant coverage factor.

In addition to the dendritic tree, 2-4 axons arise from proximal dendrites (~30 μm from the soma). The axons originate as straight, smooth processes that course beyond the dendritic field. Each axon usually bifurcates into secondary branches that give rise to thin collaterals bearing distinct boutons along their length. These collaterals extend radially from the dendritic tree for 2-3 mm within the IPL without further branching. The result is a widely spreading arborization that concentrically surrounds and is morphologically distinct from the dendritic tree, increasing the cell's overall size to ~4-6 mm in diam.

The division of this cell's processes into distinct dendritic and axonal components suggests that the axon-bearing amacrine cells may, like classical neurons, use action spikes to transmit signals over long distances in the IPL. Direct synaptic input to the cell body and thick proximal dendrites would be suitably positioned to trigger action potentials in the multiple axons and would provide a functional explanation for the unusual but characteristic location of the soma in the middle of the IPL.

450.16

MORPHOLOGICAL STUDIES OF DISPLACED GANGLION CELLS IN THE CHICKEN RETINA. G. Yang*, T.J. Millar* and I.G. Morgan. Center for Visual Sciences, Research School of Biological Sciences, Australian National University, Canberra, A.C.T. 2601, Australia.

About 4000 displaced ganglion cells (DGCs) were detected in the chicken retina by back-labelling with fast blue injected into the nucleus of the basal optic root (nBOR). Densities were slightly higher in the periphery. Soma sizes varied from 12um to 20um centrally, and from 20um to 30um peripherally. After intracellular injections of Lucifer Yellow, in flat-mount preparations 4-5 primary dendrites were seen, which ramified into highly branched and varicose fine processes. The dendritic fields could be up to 600um in diameter. In transverse section, cell bodies were located on the border of the inner nuclear layer (INL) and the inner plexiform layer (IPL), and a prominent axon descended vertically through the IPL to the optic fibre layer. The dendrites of the DGCs were unstratified in the outer part of the IPL. After double labelling for AChE, the dendrites of the DGCs were found to co-laminate precisely with the outer-most AChE-positive band which co-laminates with the dendrites of the type I cholinergic amacrine cells. Interactions between the presumptive OFF-cholinergic type I amacrine cells and the DGCs may underly the responses of the OFF-directionally selective units which have been detected electrophysiologically in the chicken nBOR.

451.1

MODELS FOR THE FORMATION OF OCULAR DOMINANCE COLUMNS: COMPUTATIONAL RESULTS. K.D. Miller and M.P. Stryker. Dept. of Neuroscience, Stanford University, Stanford CA 94305, and Dept. of Physiology, UCSF, San Francisco, CA 94143.

We have previously reported on development and analysis of a simple mathematical model for formation of ocular dominance columns in mammalian visual cortex (Soc. Neur. Abs. 12:1373 (1986)). The model provides a common framework in which a variety of activity-dependent biological models, including Hebb synapses and activity-dependent release and uptake of trophic factors, can be studied.

We report here the results of simulations. LGN laminae representing each eye are represented by 25 x 25 grids, connected via 7 x 7 arbors to a 25 x 25 grid representing the cortex (a total of 61,250 modifiable connections). If afferents within one eye are correlated in their firing over at least nearest neighbor distances on the grid, and are not anticorrelated within an arbor radius, monocular cells robustly form and are organized by intra-cortical interactions into columns. Individual afferent arbors break into patches restricted to the appropriate columns, while receptive fields refine substantially in size. The more widespread the afferent correlations within one eye, the more purely monocular the resulting cortex; positive correlation over an arbor radius yields an almost perfectly monocular cortex. The width of the columns, as determined by computing the power spectra of the resulting patterns, is accurately predicted by our previous theoretical results. The effects of monocular deprivation, modelled by reducing the activity within one eye, are accurately reproduced, and a critical period is seen.

Thus, a few simple features common to many biological models of plasticity are sufficient to account for many observed features of columnar development. Most features of the model can be analytically understood, allowing predictions of the results expected under a given plasticity model from measured biological parameters.

Supported by grants from the NIH and the System Development Foundation. Computations were performed at the San Diego Supercomputer Center.

451.2

A COMPUTATIONAL MODEL FOR THE OVERALL PATTERN OF OCULAR DOMINANCE BANDS IN STRIATE CORTEX OF CAT AND MONKEY

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In layer IV of both macaque monkey and cat primary visual cortex, geniculocortical terminals representing the two eyes are segregated into alternating zones known as ocular dominance bands. Viewed tangentially, in the monkey these bands take the form of a series of branching parallel stripes that run roughly perpendicular to the 17/18 border. On the other hand, in the cat the overall ocular dominance pattern consists of a network of irregularly branching, beaded bands, that have little if any tendency to be oriented orthogonal to the 17/18 border[1]. It has been suggested that the monkey ocular dominance pattern could result from the boundary conditions of the geniculocortical projection[2]. The present paper describes our efforts to build on this idea and formulate a single computational model that accurately predicts the quite dissimilar patterns in these two species.

Our model is based on the following observations: 1) while the LGN layers are shaped differently in these two species, the overall shape of striate cortex is the same, roughly a 2:1 ellipse; 2) in the macaque the central retina maps onto a pole of this ellipse; in the cat, it maps onto a side; 3) in both animals, neighboring points on one retina map to nearby points in the cortex, as do corresponding points from the the two retinas. Given these constraints, a computer program can generate putative cortical maps of points from the two eyes and evaluate them by computing the maximum distance between cortical representations of neighboring points (D), and the number of pairs of points that are this far apart (N). By minimizing D and N, we can determine the mapping that best meets the above constraints, and so may be called optimal. For macaque and cat boundary conditions, we obtain the following: 1) though not assumed, optimal maps invariably turn out to be retinotopic in both species; 2) in the monkey, regions representing right and left eye inputs tend to be arranged in parallel stripes, while in the cat, these regions are much less orderly and show no tendency to be oriented perpendicular to the borders of area 17.

[1]P. Anderson et al. *J. Neurosci.* in press [2]S. LeVay et al. *J. Neurosci.* 5:486–501 (1985)

451.3

MYELOARCHITECTURE OF FLAT-MOUNTED HUMAN OCCIPITAL LOBE: POSSIBLE LOCATION OF VISUAL AREA MT. M.I. Sereno, C.T. McDonald, and J.M. Allman. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Physiological and anatomical studies of extrastriate visual cortex in monkeys have uncovered a mosaic of 20 to 30 areas. The organization of these areas is remarkably similar in New and Old World monkeys, which diverged over 30 million years ago. Human extrastriate cortex is presumably organized along similar lines. However, the only visual area in humans whose borders are surely known is V1. We have therefore undertaken to describe the myeloarchitecture of human visual cortical areas using a technique for flatmounting the cortex.

Several hours after death, occipital lobes were removed and immersed in a 0.4% solution of paraformaldehyde to lightly fix the cortical grey matter but leave the unexposed white matter unfixed. The exposed surfaces of gyri in the occipital lobe were painted and numbered, and then photographed at several stages on the way to a complete flattening, which was accomplished by gently lifting off the grey matter with a blunt spatula while viewing the operation from the pial surface. The loose cortex was cut into 3 by 4 inch pieces and fixed between glass slides in 4% paraformaldehyde for several weeks before tangential 80 micron sections were cut and stained with the Gallyas silver method for myelin.

The border of striate cortex is quite apparent. A banded pattern in layer 4 of V1 with a repeat distance of about 840 microns probably represents ocular dominance columns. The rostral border of V1 is smooth except for a protruding point just dorsal to the posterior end of the lateral occipital sulcus. There is a small, ellipsoidal, coarsely-mottled, heavily myelinated area in the rostral end of the lateral extension of the parieto-occipital sulcus. This area is about 9 cm rostral to the V1/V2 border, measured on the flattened cortex, and is about 2.2 cm long dorsoventrally and 1.6 cm wide. There is no other area that is as densely myelinated within a 14 cm band surrounding the V1 border. The densely myelinated zone may correspond to area MT in non-human primates. Low contrast moving stimuli activate a similar locus as measured by PET in humans (Miezen et al., Neurosci. Abstr. 13:631). If this zone corresponds to MT, then humans have proportionally more cortex in between MT and V1 than monkeys do.

Supported by NIH grants F32 EY05887, RR07003, and the Sloan Foundation.

451.4

DYNAMIC PATTERNS OF ON-GOING COHERENT ACTIVITY IN NEURONAL ASSEMBLIES REVEALED BY REAL-TIME OPTICAL IMAGING IN CAT CORTEX. *A. Arieli* and A. Grinvald* The Weizmann Institute, IBM Research Division and The Rockefeller University, Lab. of Neurobiology, New York, NY 10021.

We combined real-time optical imaging, local-EEG and single unit recordings, to study the spatial organization and function of coherent neuronal assemblies. This report demonstrates a new approach to optical imaging that can be used to detect activity of neuronal assemblies rather than mixed populations, as well as to investigate the relationship between temporal patterns of activity of individual neurons and that of neuronal assemblies. We started by investigating on-going (spontaneous) activity (without a stimulus). The cat visual cortex was stained with the dye RH-795. The on-going activity in anesthetized cats was recorded continuously for 70 seconds using simultaneous optical (from 124 sites), local-EEG, and single unit (2 units) recordings. To search for spatio-temporal patterns of coherent activity from neuronal assemblies, we used spike triggered-averaging (spikes from a single neuron were used as a trigger for averaging). Analysis of the data revealed that the averaged optical signal, in a small patch of cortex, was well correlated in time course with the averaged local-EEG, indicating that the optical signal probably reflects mostly synaptic potentials. However, optical recordings offered better spatial resolution than local-EEG recordings. After subtraction of a common-mode slow depolarization, the remaining fast optical signals revealed a non-uniform spatio-temporal patterns in a cortical area of 2 x 2 mm. Most interestingly, we found that the amplitude of the on-going coherent activity recorded optically, per spike used for the averaging, was the same order of magnitude as that produced by activity evoked with an optimal visual stimulus. Analysis of segments of the data, grouped together according to the shape of the local-EEG, indicated that the patterns varied during the recording session (70 sec.). Therefore, these patterns must be dynamic over a short time scale. Finally, the spatio-temporal patterns appeared more heterogeneous with lighter anesthesia.

Since the amplitude of the on-going activity was so large and the patterns were dynamic, on-going activity is likely to interact in a dynamic nonlinear fashion with evoked activity. Therefore, it may play a significant role in cortical function of anesthetized and alert animals.

451.5

CENTER/SURROUND INHIBITORY INTERACTION IN MACAQUE V1 REVEALED BY REAL TIME OPTICAL IMAGING. *E. E. Lieke, R. D. Frostig, E. H. Ratzlaff*, and A. Grinvald.* IBM Research Division, and Laboratory of Neurobiology Rockefeller University, New York, NY 10021.

Previous reports have revealed stimulus specific long-range center/surround interactions in various cortical areas. However, the nature of these interactions as a function of multiple visual stimulus parameters and of the two dimensional functional organization of the cortex remains controversial. Real-time optical imaging can resolve some of the technical difficulties because it offers: (1) simultaneous recording of the intracellular membrane potential change from many sites with msec time resolution and (2) detection of subthreshold potentials.

We performed retinotopic imaging experiments in macaque V1, using 1° drifting gratings as stimuli. The optical signals were graded and spread over a cortical area larger than 6x6 mm, much larger than predicted from classical receptive field size, but consistent with the anatomical finding of long-range horizontal connections in cortex (Gilbert & Wiesel 1983). However, the spread of the optical signal might be due to electrotonic coupling of glial cells, rather than neuronal interaction. Furthermore, a positive optical signal can reflect either inhibition or excitation (Grinvald et al, Phsiol. Rev. 1988 *in press*).

To test whether excitation or inhibition is involved, we presented monkeys with four interlaced conditions, stimulating a small field or the surround alone, or both (the stimuli were centered with respect to the visual field by single unit recordings). In all cases activity related signals were detected in the entire cortical area (6x6 mm), and the analysis of the time course of the signals revealed that: (1) small (1-2°) gratings yielded activity in the "surround" cortical area that was delayed relative to the center and had a smaller amplitude. (2) A large grating with a bright "hole" (2.5-5°) produced the opposite result. (3) The two gratings (same orientation) presented simultaneously, but moving in opposite directions, yielded center and surround signals that were both smaller (~50%) and slower, relative to either grating alone. (4) Orthogonal orientations produced a similar result, but the effect was somewhat smaller relative to the former case. These results indicate that, on the average, the inhibitory effect is predominant. Additional experiments, employing more stimulus conditions are required to test if a net excitation exists between specific cortical sites (Nelson and Frost 1985, Ts'o et al 1986).

451.6

MAPPING OF EVOKED ELECTRICAL ACTIVITY IN NEOCORTICAL SLICES USING OPTICAL RECORDING TECHNIQUES. U. Kuhnt and L. Ehrenreich. Max-Planck-Inst. Biophys. Chem., D-3400 Göttingen.

Spatial and temporal changes of evoked neuronal activity were investigated in frontally cut, sensory neocortical slices (350 µm thick) of guinea pigs. Optical signals were recorded by a 10x10 photodiode array situated in the real image plane of an inverted microscope. Dependent on the objective, a single diode recorded optical changes from an area 110x110 or 70x70 $(\mu m)^2$ of tissue. Voltage sensitive absorption (RH155) or fluorescence (RH414) dyes were used. Stimulating electrodes were placed in layer I (L1) and in the white matter (WM). Stimulation in L1 evoked optical signals which were restricted to the upper three layers. The early, fast rising component of this response was due to antidromic and/or direct stimulation as could be shown by blocking synaptic transmission. Stimulation in WM evoked optical signals which could be detected in the lower five layers and spread laterally for 600-700 µm. 3-D reconstructions of amplitude distributions showed two stripes of increased activity after WM stimulation. The latencies of these stripes differed by 1 to 2 ms. The stripe with the shorter latency was localized perpendicular above the locus of stimulation, and was mainly due to antidromic activation. The second stripe was localized lateral to the stimulating electrode. It might be caused by activation of afferent fibres entering the neocortical grey matter and might reflect the basic functional unit in this cortical area.

451.7

CONNECTIVITY PATTERNS WITHIN RAT EXTRASTRIATE CORTEX REVEALED WITH THE AID OF VITAL TRACING OF CALLOSAL CONNECTIONS. R. Malach Neurobiology Dept., Weizmann Inst., Rehovot, Israel 76100.

It has been shown that rat extrastriate cortex can be subdivided into at least 9 separate areas. The connections among these areas were traced using localized injections of WGA-HRP. Accurate placement of injections was guided by the pattern of callosal connections which were revealed in vivo (Malach; Soc. Neurosci. Abs. 13:4). Results indicate that each area is connected to at least five other fields situated both in areas 18a and 18b. In all areas studied, the interconnections appear to relate similar representations of visual space. There seems to be a global map, within which the various fields are embedded, such that extreme rostral areas are connected more to lower field representations in striate and extra-striate cortex, while extreme posterior areas appear to over emphasize the upper visual field. Middle visual areas seem to be connected to all areas roughly equally. Thus, one factor distinguishing the various visual maps in the rat might be a systematic shift in emphasis of different locations within the visual field. Supported by BSF 00258 and Israel inst. for Psychobiol.

451.8

SYNAPTIC ORGANIZATION OF GABA AND GABA/TACHYKININ IMMUNOREACTIVE NEURONS IN LAYER IVCB OF MONKEY AREA 17. S.H.C.Hendry and E.G. Jones. Dept. of Anatomy & Neurobiology, University of California, Irvine, Irvine, Ca 92717

GABA- and tachykinin- immunoreactive neurons were examined electron miscroscopically in layer IVCB of normal and monocularly deprived monkeys (M. fascicularis) and in monkeys in which the geniculocortical afferent axons were labeled by anterograde degeneration. The density of axosomatic and proximal axodendritic synapses was assessed in serial thin sections. Almost half the GABA neurons were sparsely innervated (10 synapses/100 um^2) and half were richly innervated (25 synapses/100um^2). Only a small proportion of GABA cells were moderately innervated (11-25 synapses/100um^2). Most tachykinin neurons were included in that subpopulation of GABA neurons which was sparsely innervated. Sparsely innervated GABA and tachykinin neurons received a relatively large proportion of their axosomatic and axodendritic synaptic contacts from geniculocortical axons. By contrast, the richly innervated GABA neurons received a very small proportion of their contracts from these afferent axons. Following injections of tetrodotoxin into one eye, half of the GABA cells and only 10-20% of the tachykinin neurons remained immunostained. These cells were richly or moderately innervated. These findings suggest that the synaptic inputs to GABA/tachykinin neurons in layer IVCB may underlie their sensitivity to changes in visually evoked activity. Supported by NEI Grants EY 06432 & EY 07193.

451.9

A GENICULO-PRESTRIATE PROJECTION SURVIVING IN LONG-TERM DESTRIATE MACAQUE MONKEYS. Anita M. Cooper* and Alan Cowey* (SPON : J. Phillips). Dept. Expt. Psychology, Oxford, OX1 3UD, U.K.

The study investigated the distribution and morphology of neurones surviving for up to 8 years in the dorsal lateral geniculate nucleus (LGNd) after total unilateral removal of the striate cortex in a group of 11 rhesus monkeys. All surviving neurones were counted and those from a sample of 5 sections through the central LGNd were drawn and measured, and distribution through the nucleus was plotted. Neurones survived in both laminar and interlaminar zones. Cells surviving within the parvocellular layers were significantly larger than those within the "parvocellular interlaminar" zones, and those within the "magnocellular interlaminar" zones. However, there were no significant differences in cell body size between the magnocellular and parvocellular layers and the two interlaminar zones. In one animal (surviving for 96 months after the striate lesion) Horseradish Peroxidase injected into the prestriate cortex retrogradely labelled neurones throughout the degenerated caudal LGNd in topographic register with the central retina and with the position of the prestriate injection sites.

451.10

HIERARCHICAL ORGANIZATION OF RAT VISUAL CORTEX. T.A. Coogan* and A. Burkhalter (SPON: G.W. Harding). Dept. Neurosurgery & McDonnell Center for Studies of Higher Brain Function, Washington University Medical School, St. Louis, MO 63110.

We have investigated the laminar pattern of connections between visual cortical areas in the hooded rat using PHA-L tracing methods, and have found evidence for two projection types. One type terminates in layer 4 (L4) as well as in L 2/3 and deeper L1. The other is predominately to L1, deep and superficial, and also projects to L 2/3, but avoids L4. The projections from area 17 to four sites in area 18a and two in 18b, are all of the first type; projections back to area 17 from those extrastriate areas are of the second type. Likewise, the projection from area 18a to 18b is of the first type, while the reciprocal projection is of the second type. Projections of the first type have also been found to temporal and cingulate cortices.

The laminar pattern of these connections are similar to those in the primate visual cortex, where projections to areas of higher processing (forward) resemble the first type, while the reciprocal, feedback connections are like the second type. This evidence suggests that areas in the rat visual cortex are hierarchically organized similar to those in primates, with area 17 at the lowest level, the visuotopic areas within 18a next, areas of 18b higher, and the areas of temporal and cingulate cortex still higher. (Supported by NIH Grant EY05935.)

451.11

CYTOCHROME OXIDASE TOPOGRAPHY IN STRIATE CORTEX OF NORMAL AND MONOCULARLY ENUCLEATED CEBUS MONKEYS. M.G.P.Rosa*, R.Gattass and M.Fiorani Jr.*(SPON: C.G.Gross). Instituto de Biofísica Carlos Chagas Filho, Rio de Janeiro, 21941, Brazil.

The distribuition of cytochrome oxidase (CO)-rich regions was studied in flat-mounts of primary visual cortex (V1) of 2 normal and 2 enucleated Cebus monkeys. Monocular enucleation was performed under pentobarbithal anesthesia 4 to 7 months before sacrifice. In the enucleated monkey, the topographic distribution of ocular dominance (OD) stripes in layer IV was similar to that described for macaques (Le Vay et al.'85,J.Neurosci.5:486). Stripes tend to intersect the V1/V2 border perpendicularly, and to run along isoeccentricity lines throughout the calcarine cortex. In opercular V1, stripes converge and stream medially after leaving the V1 border, except at the foveal representation, where no consistent pattern is observed. In the normal monkey, the most striking aspect of CO topography in the supragranular layers was the uniformity of CO blob density. For eccentricities ranging from foveal to 60 degrees, a constant blob density (about 4 blobs/mm^2) was observed. In monocular representation, however, the blob density fell to about 2.7 blobs/mm^2 . Mean blob area decreased towards the periphery of V1. In the enucleated monkeys, CO blobs tended to be smaller in regions overlying enucleated-eye domains, but were still visible and clearly outlined, even 7 months after enucleation.
Financial support: CNPq, FINEP.

451.12

VICIA VILLOSA LECTIN IS A SELECTIVE MARKER FOR A SUBSET OF GABA CELLS IN MACAQUE STRIATE CORTEX. K.A.Mulligan, J.F.M.van Brederode* and A.E.Hendrickson. Depts. of Biological Structure and Ophthalmology, Univ. of Washington, Seattle, WA 98195.

The lectin from *Vicia villosa* (VVA) has been shown to label GABAergic neurons in both the rat (Nakagawa et al., J. Neurocytol.15:389-396) and cat (Naegele et al., Soc. Neurosci. Abstr. 13:359) cerebral cortex. We analyzed the distribution and morphology of neurons that label specifically with VVA in the macaque striate cortex. Following incubation with peroxidase-, biotin- or FITC-conjugated VVA, a population of neurons shows fine, punctate reaction product over the surface of the soma and proximal dendrites. Small numbers of labeled cells are found in layers 2, 3A, 5 and 6, but the majority (~75%) are located in a strip of cortex overlying layers 3B to 4Ca. Layers 1 and 4Cß are devoid of labeled cells. In the supragranular layers there is no clear correlation between the distribution of VVA-labeled cells and the pattern of cytochrome oxidase staining.

Double-labeling of single sections showed that most (~78%) of the VVA-labeled cells are GABA-immunoreactive. Quantitative analysis of the distribution and cross-sectional areas of double-labeled cells indicated that they make up about 30% of the total GABAergic population and include all the large (> 160 μm^2) and many medium-sized GABAergic cells. GABAergic cells with soma areas less than 60 μm^2 were not labeled with VVA. While the soma size and laminar distribution of the VVA-labeled neurons suggest that they include the large GABAergic basket cells, intracellular injections of Lucifer Yellow into VVA-labeled cells in slices of fixed cortex reveal cells with at least three distinct types of dendritic morphology.
Supported by NIH grants EY 01208, EY 04536 and EY 07031.

451.13

SIZES AND DISTRIBUTION OF GABA-IMMUNOREACTIVE NEURONS IN THE MONKEY STRIATE CORTEX. J.F.M. van Brederode*, K.A. Mulligan, R. Mehra*, and A.E. Hendrickson (SPON: C.A. Curcio). Depts. of Biol. Structure and Ophthalmol., Univ. of Washington, Seattle, WA, 98195

It has become clear that GABA-immunoreactive (GABA+) neurons in the striate cortex (SC) are not a structurally homogeneous group. In this study we describe the soma area and laminar distribution of GABA+ neurons and of a sub-population of GABA+ neurons that appear to be densely contacted by Substance P-immunoreactive terminals at the light microscopic level (GABA+/SP+). Sections of the SC were incubated in polyclonal antisera to GABA and SP, reacted with the appropriate antibodies, and visualized using different chromagens. Soma area and cell density were determined using a computer-video-microscope system. Cell density of GABA+ cells was highest in layers 2 and 4Cß. GABA+/SP+ cells were most common in layers 3, 4B, and 6, but were few in 4Cß. Soma areas (μm^2) of GABA+ cells (n=603) are shown below:

Lamina	1	2-3A	3B-4A	4B	4C	5	6
Mean	58	85	110	118	100	96	110
Range	39-90	39-181	40-198	45-265	46-269	32-218	50-265

GABA+ cells in layers 1 and 2 were smaller than those in deeper layers ($P<0.05$, Scheffe's test). Soma area variabiility was small in layers 1 and 4Cß, and large in 4B, 5, and 6. GABA+/SP+ cells were on average 1.5 times larger than GABA+/SP- cells ($P<0.05$). In conclusion, this study shows that the size of GABA+ cells in the SC varies in relation to cortical depth, and that SP+ terminals contact medium to large GABA+ cells. It is likely that GABA+/SP+ cells overlap with large GABA+ cells shown to label with the VVA lectin. This study was supported by NIH grants EY 01208, EY 04535, and EY 07031.

451.14

GAPS AND PATCHES IN VISUAL THALAMIC AND CORTICAL INPUT TO THE CLARE-BISHOP AREA. H. Vickland* and H. Sherk (SPON J. DeVito) Biol. Structure, U. of Wash., Seattle, WA 98195

Areas 17,18, and 19, as well as the LGN, all have patchy projections to the Clare-Bishop area (CB), an area of extrastriate cortex in the cat. The gaps between these patches may represent input from another source. One potential source was tested by injecting WGA-HRP into areas 17 and 18, and ^{3}H-amino acid into the lateral division of the lateral posterior nucleus (LPl), the major thalamic input to CB. There was only slight tracer spread into the medial division of LP, LPm. If input to the gaps between patches of cortical input came from LPl, we would expect the radioactive label to fall between patches of WGA-HRP. Instead, patches of the two tracers in CB were superimposed.

In a second experiment, similar but larger injections were made; both LPl and LPm were heavily involved by the ^{3}H-amino acid. While the labeled projection from areas 17 and 18 was patchy, that from LPl+LPm formed a continuous band throughout CB. The band extended beyond it, up onto the lateral bank of the suprasylvian sulcus, an area to which LPm strongly projects. This finding suggested that LPm, the tecto-recipient zone of visual thalamus, projects to the gaps between patches of input from areas 17 and 18.

Supported by EY04847 and the Alfred Sloan Foundation.

451.15

RELATIONSHIP BETWEEN THE REPRESENTATION OF THE VISUAL FIELD AND THE TOPOGRAPHY OF AFFERENT CONNECTIONS TO AREA 17 OF THE CAT.

P.A. Salin*, J. Bullier and H. Kennedy. INSERM U. 94, 16 av. Doyen Lépine, 69500 BRON, FRANCE.

We have studied the topography of the afferent connections to area 17 with double retrograde label tracing techniques. A graphic method was developped to calculate the extent of the convergence and divergence of a given connection. The convergence is the extent of an afferent structure which contains neurons converging on a small region of area 17. The divergence is the extent of area 17 innervated by a small region of the afferent structure. The results show that the convergence of the projection from the LGNd to area 17 is 0.4 mm and its divergence 2 mm. The cortical afferents present much larger values of divergence. For example, the divergences of the reciprocal connections between areas 17 and 18 are 6 mm. The divergence of the projection from area 19 to area 17 is 10 mm and those from areas 20 and PMLS are close to 20 mm. Knowing divergence and convergence and the retinotopic organizations of area 17 and the LGNd, we show that the geniculo-striate projection link regions representing the same part of the visual field. Such is not the case, however, for the cortical afferents of area 17. For example, the convergence region in area 18 represents a zone 15° wide in visual field and it converges on a projection line of area 17 which represents 5° of visual angle. These results suggest that, contrary to the geniculate afferents, the connections between the visual cortical areas preserve parameters other than retinal topography.

451.16

ORIENTATION OF INTRINSIC AXONS IN TURTLE VISUAL CORTEX. C. E. Cosans* and P. S. Ulinski (SPON: D. Margoliash). Dept. Anatomy, University of Chicago, Chicago, IL 60637.

Cells in turtle visual cortex respond to small, moving stimuli anywhere in visual space. The lateral geniculate complex receives a point-to-point representation of the retina, but projects to cortex in a point-to-line fashion. A dorsal-ventral line of geniculate cells represents points along a given vertical meridian, so all cells along the corresponding medial-lateral line of the cortex receive input from all points along that vertical meridian. This abstract deals with the way in which cortical cells receive input from all points along the horizontal meridians. Axons in visual cortex of Pseudemys scripta were labeled in an in vitro wholebrain preparation with focal HRP injections. Preparations were maintained up to 10 hours in Ringers solution, flattened and reacted with diaminobenzidine. Relatively short axons radiate from the injection sites. The directions of 306 axons relative to the rostral-caudal axis were measured in 9 injection sites. Axons project in all directions, but with some preference along the representations of the vertical meridians. Thus, one part of the visual cortex projects in all directions to other nearby parts of the visual cortex; a cortical cell apparently receives input from all points along the horizontal meridians by a series of short, intracortical projections.

451.17

INTRACORTICAL AND CALLOSAL CONNECTIVITY IN THE VISUAL SYSTEM OF THE CAT. J.P. Guillemot, L. Richer*, M. Ptito, F. Leporé. Univ. du Québec and Univ. de Montréal, Québec.

Anatomical studies have shown that corpus callosum (CC) neurons connect homo- and heterotopically the visual areas such that lower order areas project to similar or higher order areas. However, the latter are also extensively interconnected intra-cortically. The present study examined with electrophysiological methods, combined with localized inactivation, the organization of these CC connections.

Neuronal activity was recorded: from CC recipient neurons in areas 17-18 or lateral suprasylvian (LS) in split-chiasm cats, from CC fibres in normal cats; from CC fibres in split-chiasm cats. Sequential inactivation of areas 17-18 and LS was obtained with topical application of Xylocaine. Recovery of areal function was confirmed by the recovery of unit response and of the VER in the area.

Results confirmed the expected predictions: cells were driven from homotopic areas via CC. However, more complex information flow was also noted: cells projecting from 17-18 to contralateral LS first synapsed in contralateral 17-18 or ipsilateral LS. Some bidirectional activity was also seen: cells projecting via CC to LS might themselves be partially activated from the other side. The latter results, which could note be predicted from the anatomy, indicates that our method can be a powerful tool to study cortico-cortical callosal connectivity.

451.18

ACTIONS OF EXCITATORY AMINO ACID ANTAGONISTS ON SYNAPTIC POTENTIALS IN THE CAT'S VISUAL CORTEX STUDIED IN VITRO. T. Shirokawa, A. Nishigori* F. Kimura* and T. Tsumoto, Dept. Neurophysiol., Biomed. Res. Ctr., Osaka Univ. Med. Sch., Kita-Ku, Osaka 530, Japan.

Excitatory amino acids (EAAs) such as glutamate and aspartate are suggested to be transmitters at least at geniculo-cortical synapses in the cat visual cortex (Tsumoto et al. J. Neurophysiol. 55, 469, 1986). EAA receptors can be classified into three types according to affinity for agonists; N-methyl-D-aspartate (NMDA), quisqualate and kainate receptors. In the present study using slice preparations of the cat visual cortex, we addressed a question of which types of receptors mediate excitatory postsynaptic potentials (EPSPs) of layer II/III cells evoked by electrical stimulation of the underlying white matter. We used 2-amino-5-phosphonovalerate (APV) as a selective antagonist for NMDA receptors and kynurenate (KYNA) as a broad-spectrum antagonist. An application of KYNA through the perfusion medium with 0.2-1.0 mM antagonized EPSPs so that slopes of their rising phase and peak amplitudes were dramatically reduced. By contrast, APV (50µM) suppressed the EPSP slopes in some of the cells but it did not in the others. In the latter group of cells the APV-sensitive EPSPs appeared when bicuculline, a γ-aminobutyric acid (GABA) antagonist, was added to the medium. There was a tendency that the APV-sensitive excitation was induced with longer latencies and often masked by GABAergic inhibition.

451.19

ELECTROPHYSIOLOGICAL INVESTIGATION OF CALLOSAL CONNECTIONS OF RAT VISUAL CORTEX IN A SLICE PREPARATION. T. J. Teyler and R. L. Berry (Spon: D. Molfese). Dept. of Neurobiology, NE Ohio Univ. College of Medicine, Rootstown, Ohio 44272.

In vitro slice techniques have proven powerful tools for the investigation of the electrophysiology of brain circuitry in many brain areas, especially those possessing a lamellar organization. In the present study the slice preparation was used to study the circuitry of the connections between right and left hemisphere visual cortical areas which are mediated by fiber tracts passing through the corpus callosum in a curved trajectory.

Visual cortical slices were obtained from Long Evans hooded rats (2-3 wk) in a standard fashion except that curved cutting blades conforming to the callosal fiber trajectories were employed to preserve these tracts. Depth profiles of field potentials in response to white matter stimulation were recorded and subjected to current source density analysis. The curved slices greatly increased the medial extent of white matter that, when stimulated, produced current sources and sinks in cortical areas OC2MM, OC2ML, OC1M and the OC1B /OC2L border--in some cases this extended into the contralateral white matter. This finding indicated that the callosal pathway mediated these effects. The patterns of current sources and sinks evoked in these cortical areas by callosal stimulation will be presented. Supported by ONR Grant #86K0664.

451.20

CALLOSALLY AND CORTICOTHALAMICALLY PROJECTING PYRAMIDAL CELLS IN CAT VISUAL CORTEX HAVE DIFFERENT SYNAPTIC DENSITY ON CELL BODIES AND AXON INITIAL SEGMENTS. Isabel Fariñas* and Javier DeFelipe. Instituto Cajal, CSIC, Velázquez 144, 28006 Madrid, Spain.

Pyramidal cells receive only symmetrical contacts on their soma and axon initial segment (AIS) from putatively inhibitory GABAergic terminals. We have studied whether there are differences in the number of these contacts between cells projecting to different targets. Callosally and corticothalamically projecting cells in area 17 were retrogradely labelled after injections of horseradish peroxidase in the contralateral cortex or in the lateral geniculate nucleus, respectively. The AIS and portions of the somata (through a depth of about 1.75 µm at their maximum diameter, MD) of labelled cells were reconstructed from electron micrographs of serial thin sections and synapses (S) counted.

	layer	MD(µm)	S_{SOMA}	S_{AIS}
Callosal	III-IV	14-30	12-33 (n=14)	16-21 (n=5)
Corticothalamic	VI	12-22	3-12 (n=13)	1-3 (n=7)

The results indicate that these two well-defined populations of projecting cells clearly differ with regard to the synaptic density on their cells bodies and AIS. This may be a clue to the differences in their functional characteristics.

451.21

MORPHOLOGICAL TYPES OF PROJECTION NEURONS IN LAYER 5 OF RAT VISUAL CORTEX. M. Hübener* and J. Bolz. Friedrich-Miescher-Labor der Max-Planck-Gesellschaft, Spemannstr. 37-39, 7400 Tübingen, FRG.

Pyramidal cells in layer 5 of the primary visual cortex (area 17) project to several subcortical targets, such as the superior colliculus (SC), the lateral posterior nucleus (LPN) and the pons. Previous studies showed that these cells can have axon collaterals to more than one subcortical site. In area 17 of the rat there is an additional projection of layer 5 neurons to the contralateral cortex. We were interested whether different cells in layer 5 project to cortical and subcortical targets. Two fluorescent tracers, fluoro-gold and rhodamine labeled latex microspheres, were injected into the SC and the contralateral cortex respectively. Retrogradely labeled cells from the SC were restricted to layer 5. Callosal projecting neurons were found in all cortical layers near the 17/18 border and in lower layer 5 and upper layer 6 throughout area 17 . No double-labeled cells were detected, indicating that the two projections arise from different cells. There was a wide variation in soma sizes of the labeled cells. Corticotectal cells had somewhat larger cell bodies than callosal cells, but there was a considerable overlap between the two populations of neurons. In order to get a more detailed view of the cells' structure, we combined retrograde labeling with intracellular staining. After injections of microspheres into the SC or the contralateral hemisphere, brain slices were prepared from area 17 and retrogradely labeled cells were injected with Lucifer Yellow. Callosal cells have 4-6 basal dendrites an an apical dendrite terminating in or below layer 3. Corticotectal cells possess 6-9 basal dendrites and a prominent apical dendrite which always forms a large tuft in layer 1. Thus, each population of neurons has a rather stereotyped dendritic branching pattern, despite the large variation in soma size. The marked morphological differences between cortical and subcortical projecting neurons in layer 5 suggests that there is an association between structure and function of these cells.

PROCESS OUTGROWTH, GROWTH CONES, AND GUIDANCE MECHANISMS VIII

452.1

IMMUNOCYTOCHEMICAL LOCALIZATION OF GAP-43 IN THE DEVELOPING RAT BRAIN. J. W. Dani, F. H. Gage, L. I. Benowitz and D. M. Armstrong. Depart. of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

GAP-43 is a phosphoprotein synthesized in high concentrations during development of the mammalian CNS. Despite its prominent role during embryogenesis, relatively little is known of its anatomical distribution within the developing brain. In the present study, we employed immunocytochemical techniques using antibodies against GAP-43 and examined the distribution and cellular localization of this protein within brains of developing Sprague-Dawley rats. Numerous pre- and postnatal time points were examined.

GAP-43 was first detected in the periphery of whole mount preparations as early as E-10. Subsequently, dense fasicles of peroxidase reaction product could be detected throughout all prenatal time points in regions corresponding to major fiber tracts. By early postnatal days, the intensity of these labeled fibers decreased, thereafter GAP-43 immunoreactivity assumed its adult-like characteristics and could be detected within the neuropil of selected anatomical regions.

452.2

EXPRESSION OF GAP-43 DURING CNS DEVELOPMENT: AN IN SITU HYBRIDIZATION STUDY. R. Jacobson[1] and R. Neve[2], Depts. of [1]Neurology and [2]Genetics, Children's Hosp., Boston MA 02115

GAP-43 (aka B50, F1, pp46), a developmentally regulated neuronal phosphoprotein found in growth cones and synapses, is believed to play a role in axon growth and/or synaptic plasticity. If GAP-43 is important for process elongation in general, it should be expressed in most, if not all neurons during developmental axon growth. Alternatively, it might serve a specialized function in a subset of neurons. We are studying the distribution of GAP-43 message during development using in situ hybridization. Frozen sections of rat embryos at various stages are hybridized with an ^{35}S labelled RNA probe complimentary to the GAP-43 message; specific hybridization signal is detected autoradiographically. The 1.4 kb GAP-43 message is detectable on Northern blots by 14 days gestation (E14). With in situ, no signal is seen in the neural plate (E10) or in neural tube prior to the formation of the intermediate layer (E12). By E13, signal is seen in the primordial motor columns of the spinal cord, the sensory ganglia, retina, and intermediate lay layer of the brainstem, with especially strong signal in some clusters adjacent to the ventricular layer--presumably brainstem motor nuclei. Later (E15,16) much of the intermediate layer is labelled, with motor columns and patches of brainstem tegmentum showing stronger signal than surrounding areas. Thus, GAP-43 expression is widespread in early CNS development, but the level of its expression appears to be heterogenously distributed.

452.3

TYROSINE HYDROXYLASE AND GAP-43-LIKE IMMUNOREACTIVITIES IN THE DEVELOPING NIGROSTRIATAL AND MESOCORTICAL SYSTEMS. C.E. Adams, L. I.Benowitz and T.E. Finger. Dept. Cell. and Struc. Biol. Colo. Med. Sch., Denver, CO; Depts. Psych. and Neur., Harvard Med.Sch., Boston, MA

The 43 Kd phosphoprotein GAP-43(B50) has been implicated in axon elongation and synaptogenesis. In the present study, the development of GAP-43 was examined within a well characterized system to determine: 1) when GAP-43 is detectable relative to neuronal birth date and transmitter synthesis, and 2) how changes in the levels and distribution of GAP-43 correlate with developmental milestones such as initial axon elongation and synaptogenesis. Rat fetuses ranging in size from 9-19mm crown rump length were fixed and sectioned on a cryostat. The tissue was incubated in an antibody mixture containing mouse anti-tyrosine hydroxylase and sheep anti-GAP-43. Cells at the level of the mesencephalic flexure were labeled for both GAP-43 and TH at the earliest time examined (9mm). Labeling of GAP-43 was less intense in cells closer to the ventricular surface than in those nearer the pia at this stage. At later stages (13-19mm), fibers coursing towards the prosencephalon were immunoreactive for both TH and GAP-43.

452.4

EXPRESSION OF GROWTH-ASSOCIATED PROTEIN-(GAP)-43 IN THE DEVELOPING AVIAN NERVOUS SYSTEM. L. Baizer, S. Alkan*, and G. Ciment. Depts. of Pharmacology and Cell Biology and Anatomy, Oregon Health Sci. Univ., Portland, Or. 97201

Growth-associated protein-(GAP)-43 is a rapidly transported axonal membrane protein which is concentrated in the growth cone and expressed at markedly elevated levels during periods of axonal growth and regeneration in the nervous system. In order to analyze the involvement of GAP-43 in early neurogenesis and to obtain a more direct assessment of its phylogenetic conservation, a cDNA for chicken GAP-43 has been isolated and characterized. A cRNA probe corresponding to a short region of the cDNA for rat GAP-43 was used to screen a lambda gt10 cDNA library derived from embryonic day 10 chicken brain. Several positive clones were isolated and the longest (1.2 kb) was subcloned for further analysis. This cDNA hybridizes with an mRNA of about 1.5 kb which is restricted to nervous tissue in its expression and developmentally regulated in its abundance. DNA sequence analysis reveals that the predicted amino acid sequence for chicken GAP-43 displays 50-70% identity to that for rat.

The chicken GAP-43 cDNA has been subclone into a bacterial expression vector to generate antiserum to the protein. Specific probes for GAP-43 mRNA and protein will permit us to determine when and where GAP-43 begins to be expressed in the developing chicken nervous system.

Supported by grants from the NIH and the Oregon MRF.

452.5

GAP-43 PROMOTES AXONAL OUTGROWTH AND REGENERATION. R. J. O'Brian[1], R. L. Neve[2], L. Villa-Komaroff[3] and B. A. Yankner[3]. Dept. of Medicine[1], Massachusetts General Hospital, Depts. of Genetics[2] and Neuroscience[3], Children's Hospital, Boston, Massachusetts 02115.

The neuronal growth associated protein (GAP-43) is normally present at low levels in the rat pheochromocytoma cell line PC12 but is induced by treatment with nerve growth factor (NGF). In order to determine if GAP-43 is causally involved in neurite outgrowth, PC12 cells were transfected with GAP-43 cDNA in a DNA expression vector by the calcium phosphate method. Several clones were isolated that overexpressed GAP-43 RNA from the transfected cDNA and exhibited a marked acceleration of neurite outgrowth in response to NGF. The clones with higher GAP-43 RNA expression showed a greater neurite outgrowth response. PC12 cells transfected with the DNA expression vector alone showed the same neurite outgrowth response as untreated PC12 cells. Following mechanical shearing of neurites from differentiated PC12 cells, those cells overexpressing GAP-43 RNA showed a more rapid regeneration of neurites than controls; in addition, these cells displayed transient neurite regeneration in the absence of NGF. These results suggest the active involvement of GAP-43 in axonal outgrowth and regeneration.

452.6

POSSIBLE ROLE OF GAP-43 IN CALCIUM REGULATION / TRANSMITTER RELEASE. J. A. Freeman, A. A. Lettes, and B. Costello*. Department of Cell Biology, Vanderbilt University, Nashville, TN 37232.

Growth associated proteins (GAPs) may play an important role in neuronal growth and regeneration, yet very little is known about their function. We are currently investigating the role of GAP-43 on the regulation of intracellular calcium and on its possible modulation of neurotransmitter release. The PC12 clonal cell line provides an excellent system for these studies since exposure to nerve growth factor (NGF) markedly increases the expression of GAP-43.

In preliminary experiments, PC12 cells treated with NGF were loaded with the calcium sensitive dye FURA-2, and imaged with a high-resolution thermoelectrically cooled CCD camera. Cells were then incubated with GAP-43 antibody in the presence of 1% DMSO for 12 hrs. Labeling with a fluorescein tagged rabbit antibody showed that the GAP-43 antibody indeed penetrated the cells, and was sequestered preferentially into growth cones. When PC12 cells were exposed to 0.5 mM carbachol, FURA-2 fluorescence was markedly reduced compared to control (1% DMSO). Treatment with GAP-43 antibody decreased the carbachol induced release of (3H)NE by 50 %.

These results suggest a role for GAP-43 in the regulation of intracellular calcium which could effect calcium mediated events such as neurotransmitter release.

452.7

EVIDENCE FOR A SINGLE PHOSPHORYLATION SITE IN NEURONAL PROTEIN B50. P.J. Coggins* and H. Zwiers* (SPON: F. Quandt). Depts. of Med. Physiol. and Med. Biochem., Univ. of Calgary, Alta, T2N 4N1, Canada.

The neuronal protein B50 has a molecular weight of 24 kDa determined by direct chemical and cDNA sequencing, but has an apparent molecular weight of about 50 kDa on SDS gels. The protein contains 14 Ser and 14 Thr residues which are potential sites for kinase C mediated phosphorylation. Phosphoamino acid determination revealed the presence of [^{32}P]-Ser, but not [^{32}P]-Thr, in an acid hydrolysate of [^{32}P]labelled rat B50. [^{32}P]B50 was also digested by SAP and the reaction mixture was separated by HPLC, with UV and on-line beta radiation detection. The UV chromatogram contained numerous SAP products including four previously identified B50 fragments; S_1, S_2, S_3 and S_4 (McMaster et al 1988, in press). In contrast the beta chromatogram contained two labelled fragments which ran as 28 kDa and 14 kDa bands on SDS gels. Both are N terminally blocked and neither had the same HPLC retention times as the B50 fragments S_1 to S_4. Amino acid composition and partial sequence data indicated that S_1 is $B50_{204-226}$, S2 is $B50_{66-132}$ and that S_3/S_4 begin at residue 133 and extend to residue 203 or possibly further. A blocked N terminus suggested that the 14 kDa and 28 kDa SAP products are large N terminal fragments of B50. Therefore, we conclude that B50 contains a single phosphorylation site at Ser41, since all other Ser residues are eliminated.

452.8

REGULATION OF GAP-43, A NEURONAL GROWTH-ASSOCIATED PHOSPHOPROTEIN, IN PC12 PHEOCHROMOCYTOMA CELLS. B. Costello and J.A. Freeman (SPON: A. Lettes). Dept. of Cell Biology, Vanderbilt Medical School, Nashville, TN 37232.

The PC12 line of pheochromocytoma cell undergoes neuronal differentiation when grown in the presence of NGF. NGF also has been shown to increase levels of the growth-associated protein GAP-43 in PC12 cells. We have studied the regulation of GAP-43 expression by NGF as well as several other effectors under a variety of conditions. Induction of GAP-43, which is inhibited by methyltransferase inhibitors, is dependent on both NGF concentration and cell density, and exhibits some substrate specificity which appears to be related to the relative ability to support neurite outgrowth. In addition to NGF and dBcAMP, FGF also is an effective inducer, but EGF is not. Using FURA-2, we found that like NGF, FGF causes an increase in $[Ca]_i$, maximum in growth cones. Dexamethasone does not by itself affect GAP-43 levels, but appears to slightly decrease NGF induction. The effects of these various agents on GAP-43 levels correlate qualitatively with their reported effects on process outgrowth in PC12 cells and support the putative role of GAP-43 in neuronal growth.

Supported by NIH Grants EY01117 and NS18103 to JAF.

452.9

SITE SPECIFIC PHOSPHORYLATION OF GAP-43. K.F. Meiri* and L.E. Bickerstaff*. (SPON: J. Covault), Dept. Pharmacology, SUNY Health Science Center, Syracuse, NY 13210.

The growth-associated protein GAP-43 is regulated in two ways: First, its synthesis and axonal transport are induced in neurons that are growing or regenerating axons. Second, its phosphorylation is regulated even in non-growing neurons. Phosphorylation of GAP-43 may further serve to regulate its interactions with other axonal proteins, specifically those of the growth cone membrane skeleton where GAP-43 is highly enriched. We are investigating whether growth cone GAP-43 is a substrate for calcium/calmodulin kinase as well as kinase C and have established that while pure GAP-43 is phosphorylatable by both kinases, the phosphorylation sites for the two enzymes are different. GAP-43, purified from neonatal rat brains by reverse phase HPLC or calmodulin affinity chromatography and incubated with ^{32}P-ATP and either pure kinase C or pure ca/cam kinase, was partially proteolysed with trypsin and the peptides separated by reverse phase HPLC. Phosphopeptides were identified by liquid scintillation counting. Pure kinase C phosphorylated one major peptide that was different from those phosphorylated by ca/cam kinase. Amino acid analysis and sequencing of these phosphopeptides allows us to locate the phosphorylation sites within the GAP-43 molecule and will enable us to determine the phosphorylation state of membrane-skeleton-associated growth cone GAP-43. Supported by NIH Grant NS 26091.

452.10

PHOSPHORYLATION OF THE PRESYNAPTIC MEMBRANE-BOUND PROTEIN B-50 (GAP-43) BY CASEIN KINASE II. M.R. Pisano*, M.G. Hegazy*, E.M. Reimann*, and L.A. Dokas (SPON: H.J. Waller). Departments of Biochemistry and Neurology, Medical College of Ohio, Toledo, OH 43699.

B-50 (GAP-43) is a presynaptic membrane-bound phosphoprotein specific to the central nervous system. The phosphorylation state of the protein may play a role in the regulation of phosphoinositide metabolism. Of several kinases tested for their abilities to phosphorylate purified B-50, including glycogen synthase kinase 3, catalytic subunit of cAMP-dependent protein kinase, protein kinase C and casein kinase II (CK-II), only protein kinase C and CK-II were able to significantly phosphorylate B-50. B-50 phosphorylation by protein kinase C has previously been established. CK-II incorporated nearly 1 mole ^{32}P/mole B-50 and this activity was almost completely inhibited by 5 µg heparin/ml. Tryptic peptide mapping of [^{32}P]B-50 phosphorylated by CK-II showed that the radioactivity was incorporated into a single peptide. Phosphoamino acid analysis indicated that ^{32}P was incorporated exclusively into serine residues. Phosphorylation of heat-treated synaptic plasma membrane preparations by CK-II resulted in phosphorylation of several proteins but the major substrate was B-50. These studies indicate that B-50 could be a physiological substrate for CK-II. Supported by NIH grants NS 23598 and DK 19231.

452.11

IDENTIFICATION OF A NOVEL NEURONAL INTERMEDIATE FILAMENT PROTEIN THAT IS PHOSPHORYLATED IN PC12 CELLS. J. M. Aletta[1], R.H. Angeletti[2], R.K.H. Liem[1], C. Purcell*[2], M.L.Shelanski[1], and L.A.Greene[1]. [1]Dept. of Pathology Columbia University N.Y.C. N.Y. 10032 [2]Div. Neuropath. Univ. of Pennsylvania, Phila. PA 19104.

NGF induces an mRNA in PC12 cells that encodes a novel neuronal intermediate filament protein that is present in PNS and certain CNS neurons (Leonard et al. J. Cell Biol. 106:181, 1988). The present results concern the identification and characterization of this protein. Partial microsequencing of the major cytoskeletal protein of PC12 cells (58 kDa; pI=5.6-5.8), derived by extraction with 1% Triton X-100, yielded a 14 residue sequence that is identical to a portion of the sequence deduced from the NGF-induced message, but not to sequences of other known proteins. Also, an antiserum raised against a 19 residue synthetic peptide corresponding to the unique C-terminus region of the protein encoded by the NGF-regulated message specifically recognizes the 58 kDa protein on Western blots and, by immunofluorescent cell staining, filamentous structures as well as a dense perinuclear cap. The protein resolves as 4 to 5 spots by IEF and is elevated in cytoskeletons from NGF-treated cells relative to those from non-treated cells. Two of these isoforms are found in the Triton-soluble fraction. Isoforms associated with the cytoskeleton are phosphorylated, but the Triton-soluble forms are not metabolically labeled with ^{32}P-orthophosphate. Relative phosphate incorporation is elevated within 2h of exposure to either NGF, TPA, a cAMP analog or 40mM K^+. This protein and its phosphorylation may be involved in NGF-mediated neurite outgrowth and/or stability.

452.12

TUBULIN TYROSINE AND SERINE PROTEIN KINASES IN RAT BRAIN GROWTH CONES. N. Cheng* and N. Sahyoun. (SPON: S. Burgess). The Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709.

We had previously described the multi-site phosphorylation of growth cone tubulin on ser and tyr residues. We now present data pertaining to the protein kinases catalyzing these reactions. The Triton X-100-soluble fraction of growth cone membranes contained a ser protein kinase which preferentially phosphorylated the β-subunit of tubulin. The enzyme was retarded on several gel-permeation columns, displayed polydisperse behavior, and thus could be separated from the majority of growth cone polypeptides. The partially purified enzyme phosphorylated tubulin with an apparent K_m of 0.4 µM, and the corresponding values for ATP and $MgCl_2$ were about 23 µM and 2.5 mM, respectively. The presence of a tyr tubulin protein kinase in the cytoskeletal fraction of growth cones was further confirmed by the selective binding of anti-phosphotyrosine antibodies to phosphorylated cytoskeletal tubulin following electrophoretic transblotting. The cytoskeletal fraction also phosphorylated poly glu-tyr efficiently with an apparent K_m for ATP of 33 µM. Moreover, affinity labeling of the cytoskeletal enzyme preparation with (α-^{32}P) 8-azido ATP labeled specifically a unique polypeptide with M_r value of 49,000. The relationship of the ATP-binding protein to the protein kinase is under investigation.

452.13

EFFECTS OF INHIBITORS OF CA2+ -ACTIVATED NEUTRAL PROTEASE (CANP) AND PROTEIN KINASE C (PKC) ON NEURITE OUTGROWTH. L. HSU, A.Y.JENG* and K.Y.Chen*, Biol.Dept, Seton Hall Univ.,South Orange NJ 07079; Research Dept.,Ciba-Geigy Corp.,Summit,NJ 07901;Chem.Dept., Rutgers Univ.,Piscataway NJ 08854.

Upon activation by 12-0-tetradecanoylphorbol-13-acetate (TPA) or diacylglycerols, PKC is further activated by CANP to liberate a catalytic fragment which phosphorylates other cytosolic proteins to generate physiological responses. We have previously reported that activators of PKC promoted neurite outgrowth from ganglia explants. To further substantiate the involvement of PKC in this system, the effects of a selective inhibitor of CANP, leupeptin, and a potent inhibitor of PKC, staurosporine on neurite outgrowth were examined. Pretreatment with 20-500 uM leupeptin significantly reduced the formation of thick neurite fascicles induced by TPA. Leupeptin itself did not have any effect on neuronal development. Staurosporine inhibited PKC in vitro with an IC50 of 3 nM. At concentrations of 10-200 nM, it was found to exhibit neurite-promoting activities which were additive to those of TPA and were not inhibited by pretreatment with leupeptin. These results suggest that the activation of PKC by CANP is important in the induction of neurite outgrowth. While staurosporine is a potent inhibitor of PKC in vitro, its effect in vivo appears to be complicated by its non-specific nature towards other protein kinases. (Supported by NS21262,NIH).

452.14

PROTEASES IN DEVELOPING CHICK RETINA: RETINAL CELLS DIGEST SUBSTRATE BOUND GELATIN IN VITRO. J. B. Sheffield and D. Graff, Temple University, Philadelphia, PA. 19122

During retinal histogenesis, cells and their extensions migrate through the tissue to final positions. We report initial evidence that proteolytic activity might be associated with this process. Three high molecular weight bands of gelatinolytic activity can be found in zymogels of homogenates of embryonic chick and calf retinas, and in media from cultured retinal cells. Prior incubation with 0.1mM 4-aminophenyl mercuric acetate, a thiol blocking agent, enhances activity in gels and soluble assays. 5 mM 1,10 phenanthroline inhibits.

Gelatin was conjugated to rhodamine through an isothiocyanate bridge. 75 µl of a .1 mg/ml solution was applied to cleaned cover slips and allowed to dry. The slips were placed in T-25 culture flasks, and 5 ml of a suspension of 1.5 x 10^5 freshly dissociated 14 day embryonic chick retina cells/ml was added. Cultures were maintained for periods of up to one week, and samples were taken at various times for fluorescence microscopy. After periods of up to 24 hours, little change was seen. Three days after the culture was initiated, dark patches were visible in the fluorescent substrate. Two patterns were observed. The first is a restricted patch, following the path of a neuron and its growth cone. The second is more diffuse, and corresponds to the leading edge of a flat cell.

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452.15

ENZYME BASED NEURITE PROMOTION BY SENSORY NEURONS IN VITRO. R.J. Riopelle and K.E. Dow. Queen's University, Depts. of Medicine, and Peds, Kingston, Canada K7L 3N6.

Enzymatic mechanisms have been proposed for neurite extension in development and consensus enzyme systems have been identified. Neural crest cells at early embryonic stages of avian development possess cell surface enzyme systems that may play a role in migration. We have recently determined that avian embryonic sensory neurons from ED 8 chick possess a cell surface galactosyltransferase (GalTase) activity that is involved in neurite formation on a laminin substrate. The acceptor substrate for GalTase is N-acetylglucosamine (GlcNac). In the presence of GlcNac or the related polymer chitotriose there was a dose dependent inhibition of neurite outgrowth by sensory neurons on the laminin substrate. Furthermore dose dependent inhibition of neurite outgrowth was also observed in the presence of a competitive acceptor substrate - alpha lactalbumin, and in the presence of the catalytic substrate for GalTase-UDP galactose (UDPgal). Since UDPgal is not normally found in the extracellular space, cell surface GalTase may provide a neurite promoting mechanism based soley on adhesion mechanisms. It remains possible however that sensory neuron GalTase enzymatic activity could modulate the extracellular milieu during the phase of patterned cell death at which time the catalytic substrate UDPgal might be released to the extracellular milieu.

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452.16

THE PROTEASE TRANSIN IS INDUCED PRIOR TO NEURITE EXTENSION IN NGF-TREATED PC12 CELLS. C.M. Machida* and G. Ciment. (SPON: D. Trune). Department of Cell Biology & Anatomy and the Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

We report here that transin mRNA levels increased dramatically in rat pheochromocytoma (PC12) cells upon stimulation with nerve growth factor (NGF), reaching their maximum level within 24 hours. Although neurofilament (NF-M) mRNA levels also rose with the same time course in NGF-treated PC12 cells, the basal (uninduced) levels of mRNA for this cytoskeletal component were also high. When we compared these biochemical data with the morphological data, we found that transin mRNA levels rose prior to the initial appearance of neurites. Epidermal growth factor (EGF) and basic-heparin binding growth factor (b-HBGF) were unable to elicit both an induction in transin mRNA levels as well as a change to the neuronal phenotype. We also report that transin mRNA induction is not a primary effect of NGF, since cycloheximide was able to block the NGF induction. These data suggest that transin induction is part of the constellation of events induced upon NGF treatment, and may be involved in the process of neurite extension.

452.17

SODIUM CHANNELS ACCUMULATE ON AXOLEMMA OF HYPEREXCITABLE NEUROMA AFFERENTS M. Devor[1], C.H. Keller[*2], T. Deerinck[*3], S.R. Levinson[4] and M. Ellisman[3] [1]Life Sciences Institute, Hebrew Univ., Jerusalem 91904, Israel, [2]Scripps Institute Oceanography, and [3]Dept. Neuroscience, UCSD, LaJolla, CA. 92093, [4]Dept. Physiology, Univ. Colorado, Denver CO. 80262

Afferent axons caught in a nerve end neuroma following nerve section in vivo frequently develop ectopic impulse generating capability. We show that the emergence of such electrical hyperexcitability is associated with increased Na^+-channel content. Acute section of the lateral line nerve in the weakly electric Gymnotid fish Apternotus leptorhyncus silenced electroreceptive nerve afferents. Recordings made 10-21 days later yielded high frequency ongoing rhythmic firing independent of the electric organ discharge, which was eliminated on re-section. Single fibers microdissected from such neuromas were stained with a highly specific polyclonal antibody raised in rabbits against purified Na^+-channel protein from eel electroplax organ. Controls included deletion of primary, and use of antibody preabsorbed with purified Na^+-channel protein. Rhodamine-coupled GAR secondary antibody visualized heavy, specific labeling of preterminal demyelinated segments and terminal endstructures, and PAP immuno-electron microscopy confirmed antigen localization to the axolemma. We suggest that this Na^+-channel accumulation may account for neuroma hyperexcitability including related positive sensory symptoms in traumatic neuropathies.

452.18

L TYPE CALCIUM CHANNELS MAY REGULATE NEURITE GROWTH IN CHICK EMBRYO BRAIN NEURONS. M. Lomme,* C. Ferguson,* D. Shugarts,* J. Rosack,* P. Caracciolo,* T. Gisi,* P. Nichols,* T. Audesirk and G. Audesirk. Biology Dept., University of Colorado at Denver, 1200 Larimer St., Denver, CO 80204.

The intracellular free calcium ion concentration is thought to regulate the growth of neurites in several types of cultured neurons. Calcium influx through voltage-sensitive calcium channels increases the intracellular calcium concentration and therefore may influence the rate of neurite growth.

In vertebrate neurons, the L, T, and N calcium channels respond differently to heavy metals (Cd^{2+} and Ni^{2+}) and to organic compounds such as verapamil, the dihydropyridines, amiloride, and the aminoglycosides. In primary cultures of neurons from chick embryo brains, L channel blockers, such as Cd^{2+} (10 uM) and nifedipine, dramatically reduce neurite growth. Neither T channel blockers, such as Ni^{2+} (up to 100 uM), nor N channel blockers, such as most aminoglycosides, affect neurite growth. Streptomycin, which has been reported to block N type channels at low concentrations and L type channels at high concentrations, blocks neurite growth only at very high concentrations. Therefore, calcium influx through L type channels appears to promote neurite growth in these neurons.

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452.19

PATCH-CLAMP OF CULTURED CRUSTACEAN PEPTIDERGIC NEURONS. D. Meyers, R. A. Graf, P. Ruben and I. Cooke. Békésy Laboratory of Neurobiology and Department of Zoology, University of Hawaii, Honolulu, HI 96822.

The neurosecretory cells of the crab (Cardisoma carnifex) isolated and cultured in simple, defined media, show immediate, vigorous outgrowth and retain their biochemical distinctness (Graf, et al., this vol.). We report here that they also retain the voltage-dependent currents expected from in situ studies; Na-current is lacking as expected in the absence of the axon. Membrane potentials recorded with microelectrodes are -30 to -60mV, often with random, slow fluctuations of ca. 5mV. Action potentials were not observed. Whole-cell patch electrodes at the soma were used to voltage-clamp large (>20um) cells having broad lamellipodial or radial outgrowth. During the first 5d after plating, outward currents predominate. These include a rapid-onset, time- and depolarization-inactivated current attenuated by 4AP and a slowly developing, non-inactivating outward current attenuated by TEA. Inward current recorded under regimes which minimize K and Cl currents was unaffected by TTX but rapidly blocked by Cd (0.2mM), and is thus attributed to Ca. Ca current is unchanged by V_h between -40 and -60mV, observable for steps to >-30mV and maximal at +10mV (up to 200pA). Minimum time to peak is ca. 5ms. Decay during steps to +10mV shows an initial rapid phase (<10ms) and a much slower one (>50ms); at 160ms, >50% of peak current remains. Inhibition of Ca-currents, peptide secretion and outgrowth by Cd is consistent with our suggestion that outgrowth may utilize secretory mechanisms including Ca-mediated exocytosis.

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452.20

THE ORGANIZATION OF MYOSIN IN CULTURED NERVE GROWTH CONES DETECTED BY IMMUNOELECTRON MICROSCOPY. P.C. Bridgman and M.E. Dailey. Dept. of Anatomy and Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110

A postembedment labeling procedure using rapid freezing, freeze substitution and low temperature embedding in Lowicryl K11M has been adapted for the detection of myosin in cultured rat superior cervical ganglion nerve growth cones. This was necessary because conventional fixation techniques for immunoelectron microscopy either distort the structure of growth cones or destroy the antigenicity of myosin. An antiserum specific for the heavy chain of human platelet myosin was used for labeling. Rotary shadowing of purified platelet myosin/antiserum mixtures showed antibody binding at several sites along the tail and head portion of the molecule. Cross-reaction of the antiserum with the rat nerve cells was confirmed by immunoblots and immunofluorescence of frozen, freeze substituted cultures. Immunoelectron microscopy using colloidal gold showed a diffuse random distribution of label in broadly spread lamellipodia. Aggregates of label were seen at the base and along the length of filopodia and in the central region of the growth cone. The aggregates were often associated with distinct irregularly shaped areas of increased electron density. Label was not consistently associated with distinct filaments, although the relatively light staining of the sections would probably prevent detection of small filaments. The distribution of the myosin label suggests that myosin may play a role in growth cone motility.

(Supported by NIH grant NS15070.)

453.1

LOCALIZATION OF 3070 IMMUNOREACTIVITY IN DEVELOPING CHICKS: RELATIONSHIP TO NEURAL EXPRESSION BY CELLS DERIVED FROM THE NEURAL CREST. H.D. Pomeranz, R.F. Payette*, N.R. Smalheiser+ and M.D. Gershon, Department of Anatomy and Cell Biology, Columbia University, P & S, New York, NY 10032 and +Department of Pediatrics, The University of Chicago, Chicago, IL 60637.

Laminin facilitates cellular attachment and neurite outgrowth. A 67 kDa laminin receptor has been implicated in attachment. Another laminin-binding protein has been identified, M_r 120 kDa ("cranin"; Smalheiser and Schwartz, 1987) or 110 kDa (Kleinman et al., 1988), which may be a neural laminin receptor. An antiserum, 3070, was raised by immunizing rabbits with the 120 kDa protein. The antiserum demonstrates the major HNK-1-immunoreactive protein of embyonic chick brain. Sites of 3070 immunoreactivity (IR) in aldehyde-fixed chick embryos were examined immunocytochemically along with markers to identify neural crest-derived cells (monoclonal antibody, NC-1) and neuronal precursors (antibodies to neurofilament-M [nf] and an associated protein [NAPA-73]). The earliest localization of 3070-IR, at stage 10, was in the neural tube. At this time, virtually all neural tube cells expressed 3070-IR, but only a small fraction expressed NC-1-IR and these did not co-express 3070-IR. No 3070-IR was found in the premigratory neural crest; however, at later stages (19-24) NC-1-immunoreactive cells that co-expressed 3070-IR were found migrating along the aorta, between the mesonephri and within the gut. The neural tube, notochord, dorsal roots and mesonephric tubules also displayed 3070-IR. Nf expression was not detected in the 3070-IR cells until stage 26. Many nf-IR cells in the branchial arches failed to express 3070-IR or NC-1-IR. At later stages in the gut 3070-IR was expressed on a subset of NC-1-IR cells and a subset of the 3070-IR cells also expressed nf-IR. These observations are consistent with the hypothesis that crest-derived cells do not acquire neural laminin receptors until they begin to differentiate as neurons. Supported by NIH grant # NS 15547.

453.2

COEXPRESSION OF AUTONOMIC AND SENSORY NEUROTRANSMITTERS BY CULTURED AVIAN NEURAL CREST CELLS. G.G. Leblanc. Developmental Biology Center, Univ. of Calif., Irvine, CA 92717.

Neural crest cells give rise to both autonomic and sensory neurons. It has been proposed that the precursors of these two kinds of neurons become segregated into separate lineages very early in development, possibly even before the onset of neural crest cell migration (Le Douarin, N. M., *Science*. 231:1515, 1986). This idea was based in part on examinations of the neurotransmitter phenotypes expressed by avian neural crest cells developing in vitro under different kinds of culture conditions. It was reported that neural crest cells grown in serum-free medium gave rise to substance P-immunoreactive cells, but not to tyrosine hydroxylase (TH)-immunoreactive cells. In contrast, neural crest explants cultured in medium containing serum and chick embryo extract contained many TH-immunoreactive cells, but were devoid of substance P containing neurons. Based on these results, it was proposed that the premigratory neural crest contains separate populations of sensory and autonomic neuron precursors which have different requirements for survival in vitro (Ziller et al., *Dev. Biol.*, 120:101, 1987). However, we have observed extensive differentiation of substance P-immunoreactive cells in cultures containing both serum and chick embryo extract by five days in vitro. Moreover, double labelling experiments showed that many of the substance P-immunoreactive cells in these cultures also contained tyrosine hydroxylase. These results indicate that individual neural crest cells can express both sensory and autonomic neurotransmitters.

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453.3

DEVELOPMENTAL POTENTIAL FOR OLIGODENDROCYTES AND ASTROCYTES IN CULTURED EMBRYONAL CARCINOMA CELLS. L. Bambrick*, J. DiMichele*, J. Bell* and P. Braun. Dept. of Biochemistry, McGill University, Montreal, P.Q., H3G 1Y6 (sponsored by L. Bernier).

Embryonal carcinoma (EC) cells are pluripotent stem cells; the P19 EC cell line can be induced by treatment with retinoic acid (RA) to differentiate in vitro into CNS cells. These include neurons, fibroblasts and astrocytes (AC's). Oligodendrocytes (ODC's) have not been observed nor has it been shown that the AC's seen in RA-treated P19 EC cells include Type 2 AC's. These cells are reported to share a common bipotential O-2A progenitor with ODC's. We investigated whether the failure of P19 cells to produce ODC's was due to the absence of the O-2A progenitor and whether conditions known to stimulate ODC proliferation in vitro would affect the developmental outcome in P19 EC cells. Immunocytochemistry was used to characterize cultured cells as Type 1 AC's; $GFAP^+$; Type 2 AC's; $GFAP^+/A_2B_5^+$; ODC's; $GalC^+$. We report the presence of both Type 1 & Type 2 AC's and confirm the absence of ODC's in RA-treated P19 EC cells. Soluble rat brain extracts induced a limited differentiation of P19 EC cells into ODC's. This activity was greatest in extracts from 7 day old animals, a time of high ODC proliferation in vivo and of peak brain extract-induced ODC proliferation in vitro. Supported by the Alberta Heritage Foundation for Medical Research and the MS Society of Canada.

453.4

MULTIPOTENT PRECURSOR CELLS IN XENOPUS CILIARY MARGIN: A CELL LINEAGE ANALYSIS. Richard Wetts, George Serbedzija, and Scott E. Fraser. Dept of Physiol & Biophysics and the Developmental Biology Center, Univ Calif, Irvine, 92717.

Previously, we used cell autonomous lineage tracers to show that single Xenopus optic vesicle cells can give rise to all major cell types, including glia. We present here observations on the multipotency of ciliary margin (CM) cells. The CM is a proliferative zone that contributes new cells to the peripheral retina. It is possible that CM cells are multipotent, like optic vesicle cells, or that each cell could be committed to forming a specific type of retinal cell. Rhodamine dextran (LRD) was injected into single CM cells (St 39), and the animals were fixed (St 50).

In a prospective lineage study, the developmental potential of the injected precursor is determined by identifying the different types of cells into which its descendants have differentiated: I) In 30 of our 36 clones, the LRD-labeled cells were distributed over all 3 retinal layers; this indicates that CM cells are multipotent. II) All major cell types, identified by their morphology when the LRD filled their processes, were found within clones that included other identified cells. At least 4 clones included all major cell types, and even small clones located in 1 or 2 layers appeared to consist of multiple types. These observations indicate that CM precursor cells are not restricted in the types of cells which they can produce. Thus, our data suggest that the molecular events which regulate the commitment and differentiation of specific cell types occur relatively late in retinal lineages.

Supported by NSF, McKnight Found. and Monsanto Corp.

453.5

DORSAL ROOT GANGLION X NEUROBLASTOMA HYBRID CELLS EXPRESS NEURONAL ANTIGENS. R. Dingledine and L.M. Boland, Curr. Neurobiology and Dept. Pharmacology, Univ of North Carolina, Chapel Hill, NC, 27599.

Immunocytochemistry was used to study the expression of neuronal and DRG-like antigens by the F-11 hybrid cell line (Platika et.al., PNAS, 1985). F-11 cells were stained using a monoclonal antibody to neurofilament protein, but did not stain for glial fibrillary acidic protein nor fibronectin. A number of monoclonal antibodies recognize functionally distinct subpopulations of adult DRG neurons. Not all F-11 cells expressed surface carbohydrates recognized by SSEA-3, SSEA-4, or B2. Staining for each antibody was assessed by counting stained and unstained cells and classifying each cell based on its morphology. SSEA-3 stained about 20% of the hybrid cells, while 40-60% were stained by SSEA-4 or B2. The percentage of cells stained by SSEA-3 was greatest for cells that were either flat or multiform and lowest for cells that had a round cell body or a spindle shape. In contrast, the staining by SSEA-4 or B2 was more homogeneous for different morphological cell types. Staining for all antibodies used was not different for hybrid cells fed with growth medium or differentiation medium for 2-3 days. The neuroblastoma parent (N18TG-2) of the hybrid cells did not stain with any of these antibodies. These results suggest that F-11 cells are derived from a neuronal parent and express surface antigens found on sensory neurons, but do not homogeneously express characteristics of any particular subpopulation of DRG neurons. (Supported by NS23804)

453.6

GLUCOCORTICOID-INDUCED PNMT-IMMUNOREACTIVE CELLS IN THE SUPERIOR CERVICAL GANGLION OF THE RAT. H. Paivarinta[1], L. Eranko[2], V.M. Pickel[1] and T.H. Joh[1]. [1]Dept. Neurology, Cornell Univ., New York, NY 10021, [2]Dept. Anatomy, Univ. Helsinki, Helsinki, Finland

Light and electron microscopic immunocytochemistry were used to study the effect of glucocorticoids on the development of phenylethanolamine-N-methyltransferase (PNMT)-immunoreactive cells in the superior cervical ganglion of early postnatal rats. Newborn rats were daily injected with hydrocortisone acetate on postnatal days 2-6. Already 6 hours after the first glucocorticoid injection, some PNMT-immunoreactive cells and fibres were seen in the ganglion. After 5 days the number of PNMT-immunoreactive cells was increased and long processes and fibre networks were seen. Ultrastructurally, most of the PNMT-immunoreactive cells had an appearance of SIF cells: heterochromaffin clumps along the nuclear envelope and in the center of the nucleoplasm, and dense core vesicles. Also SIF cells, non immunoreactive to PNMT antiserum, were seen. Some PNMT-immunoreactive cells had the ultrastructural characteristics of nerve cells. They had a voluminous cytoplasm, dispersed nuclear hererochromaffin, and no granular vesicles. These results demonstrate that the glucocorticoids induce PNMT immunoreactivity both in SIF cells and cells with characteristics of nerve cells. Supported by NIH grant 1P01MH 42626-01.

453.7

DETERMINATION OF THE LENGTH OF THE CELL CYCLE AND THE DNA-SYNTHETIC PHASE FOR THE PROLIFERATING CELLS IN THE DENTATE GYRUS OF C57BL/6J MOUSE USING BROMODEOXYURIDINE IMMUNOHISTOCHEMISTRY. S.B. Lewin*, M.W. Miller, and R.S. Nowakowski. Dept. of Anatomy, UMDNJ-Robert Wood Johnson Medical School, and UMDNJ-School of Osteopathic Medicine, Piscataway, NJ 08854.

As part of a quantitative analysis of cell proliferation in the developing dentate gyrus, we have determined: 1) the length of the cell cycle, 2) the length of the DNA-synthetic phase (S-phase), and 3) the growth fraction (i.e., the proportion of cells in the hilus that comprise the proliferating population). A cumulative labeling procedure was used. At postnatal day 20, C57BL/6J mice were injected with bromodeoxyuridine (BUdR) at two hour intervals for a total period of 12 hours. Animals were sacrificed at selected intervals, and the brains were processed for immunohistochemistry using an antibody directed against BUdR. Sections from the approximate middle of the septo-temporal axis of the hippocampal formation were selected, and the number of BUdR-labeled and unlabeled cells in the hilus of the dentate gyrus counted. The number of BUdR-labeled cells increased linearly for 9 hours from an initial value of 12% of the total number of cells to a maximum value of 24% of the total. Calculations from these findings indicate that at this age the cell cycle is 18 hours, the S-phase is 9 hours and 24% of the cells in the dentate hilus are part of the proliferating population. In addition, the linear increase in the proportion of BUdR-labeled cells indicates that, at least in terms of the lengths of the cell cycle and the S-phase, the proliferating cells in the dentate hilus at this age comprise a single population.

Supported by NIH Grants NS 23647, AA 07568, AA 06916, DE 07734 and a grant from the UMDNJ Foundation.

453.8

IMMORTALIZATION OF AN OLIGODENDROCYTE PRECURSOR CELL VIA A RETROVIRUS CARRYING A TEMPERATURE SENSITIVE ONCOGENE. G. Almazan* and R. McKay. Departments of Biology and Brain Sciences, E25-435, Whitaker College, MIT, Cambridge, MA 02139.

Oligodendrocytes, the myelin-forming cells of the CNS, develop during the first week of postnatal life in the optic nerve of the rat. Their progenitors are bipotential cells which also give rise to type 2 astrocytes. To obtain permanent cell lines, primary cultures from the optic nerve of 3-d-old S.D. rats were infected with a retrovirus vector transducing the tsA58/U19 combination large SV40 T oncogene. At the permissive temperature, one of the derived cell lines proliferates and expresses the T antigen and small amounts of GC (galactocerebroside), a surface lipid marker for oligodendrocytes and A_2B_5 (surface gangliosides). At the non-permissive temperature, the cells lose the T antigen expression, stop proliferating, and stain very strongly with GC and A_2B_5 antibodies. In addition, the two main protein components of myelin, MBP(myelin basic protein) and PLP (proteolipid protein) are also expressed.

These results show that a precursor cell line spontaneosly differentiates into a mature oligodendrocyte when the immortalizing oncogene is inactivated. The functionality or potential use as replacement therapy of these cells will further be explored in transplantation experiments using myelin deficient mutant rodents.

453.9

RETROVIRAL VECTORS USED TO STUDY CELL LINAGE IN THE CEREBRAL CORTEX. B.P. Williams, L. Thurlow, R. Moore, J. Price, (SPON: J. Brockes) Laboratory of Embryogenesis, NIMR, Mill Hill, London, NW7 1AA, U.K.

We have developed a method for studying cell lineages which uses a retroviral vector, called BAG, to genetically mark embryonic cells in vivo (Price et al. (1987) PNAS 84: 156-160). BAG carries the bacterial β-galactosidase gene which can be detected histochemically, in infected cells, using the substrate X-gal.

We have injected BAG into the cerebral vesicles of rats in utero on embryonic day 16. The virus infects the ventricular cells of the developing cerebral cortex. The virus used is of such a titre that infection is a rare event. Analysis of the resultant clones allows us to ask two types of question. Firstly, how the different neural cell types are related; secondly, how do the progeny of a single progenitor cell expand in time and space to contribute to the cortical structure. In preliminary studies we have defined two types of progenitor cell in the developing cortex, one that gives rise to astrocytes but not neurones and one that gives rise to neurones and some types of glial cells. Further, we infected embryonic cortical cells in culture and have been able to mark clones similar to the ones we have defined in vivo. This tissue culture system allows us to ask what factors influence the fate of multipotential progenitor cells.

453.10

A STRAIN AND SPECIES SPECIFIC MONOCLONAL ANTI-NUCLEAR ANTIBODY AS A CELL MARKER FOR MOUSE CHIMERAS. R. J. Mullen and T. Cichocki*. Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

With the aim of producing species-specific monoclonal antibodies that could be used as cell markers in chimeric mice, BALB/c mice were immunized with cell nuclei isolated from Mus caroli brains. A monoclonal antibody (IgG) was produced that appears to bind the nuclear membrane of Mus caroli, but not BALB/c, mice. Sections of formaldehyde-fixed, polyester wax embedded brain, were immunohistochemically stained using Vector ABC with HRP. The resulting staining of nuclear membranes gives the appearance of rings, hence, the antigen has been nicknamed "ringo". The epitope recognized by this antibody was present not only in Mus caroli but also in Mus castaneus as well as in all inbred strains of laboratory mice tested including C57BL/6J, C3H/HeN, DBA/2, SWR, AKR and LPT. Since the antibody stained C57BL/6 (B6) nuclei but not BALB/c nuclei, seven CXB (i.e., BALB x B6) recombinant inbred strains were examined and the observed strain distribution pattern matched perfectly with the Gpd-1 locus on the distal end of Chromosome 4. B6 congenic mice carrying the BALB/c allele of the H-18 locus on Chromosome 4 (i.e., B6.C-H-18c) were ringo(-), suggesting the gene controlling this antigen is located on the distal end of chromosome 4 near the Gpd-1 and H-18 loci. In brain sections, the antibody appears to recognize many if not all cell types. The staining is particularly strong in the cerebellum. Preliminary observations suggest ringo may be specific to neural tissue and may first become evident sometime after postnatal day 10. We have recently examined a C3H<->BALB chimera and mosaicism was evident among cerebellar granule cells and Purkinje cells as well as in the dentate gyrus and hippocampus. (Supported by NIH Grants NS16156 and EY07017)

453.11

CHIMERIC ANALYSIS OF LINEAGE RELATIONSHIPS AMONG MOUSE LUMBAR MOTONEURONS. M.W. Vogel, A.W. English, and K. Herrup. Dept. of Human Genetics, Yale Med. School, New Haven, CT 06510 and Dept. of Anatomy, Emory Univ., Atlanta, GA 30322.

We have analyzed mouse chimeras to address two questions about the role of lineage relationships in the development of the lumbar lateral motor column: 1) are all lumbar motoneurons descended from a defined group of progenitor cells, and 2) is motoneuron lineage related to motoneuron connectivity, as defined by muscle innervation? We have identified medial gastrocnemius motoneurons by HRP backlabeling of the muscle nerve in β-glucuronidase chimeras, and employed TMB-DAB and β-glucuronidase histochemistry to analyze both motoneuron identity and genotype. The labeled motoneuron pools in 4 chimeras contained cells of both genotypes, indicating that a motoneuron pool is not a descendent clone of a single progenitor. The genotype ratios of labeled motoneurons differed from the genotype ratios of total lumbar motoneurons, suggesting that medial gastrocnemius motoneurons are not randomly selected from an uncommitted population of lumbar motoneurons. The genotype ratios of lumbar motoneurons in the 4 labeled and 1 unlabeled chimera also varied systematically between animals and between the right and left sides of individual chimeras. This data suggests that lumbar motoneurons are descended from a smaller set of progenitor cells and that the progenitors are selected independently on the right and left sides of the spinal cord.

Supported by the NIH (MWV:NS-06789; AWE:NS-20545; KH:NS-20591 and NS-18381), and the March of Dimes Birth Defects Foundation (KH).

453.12

AN ANTIBODY TO RETINAL GANGLION CELLS RECOGNIZES PREMIGRATORY AND MIGRATING CELLS IN THE DEVELOPING CHICK RETINA. Steven C. McLoon and Roxann Severson*. Dept. of Cell Biology & Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

We have developed a monoclonal antibody, RA4, that recognizes retinal ganglion cell axons in the mature retina. Between embryonic days 3 and 9, the RA4 antigen was associated with cells in certain regions of the retina in addition to the optic fiber layer. The RA4 positive cells were of three types: an apolar cell adjacent to the ventricular surface, a bipolar cell that spanned the thickness of the retina and a monopolar cell in the ganglion cell layer. Evidence suggests that these cells are premigratory and migrating retinal ganglion cells. The expression of the RA4 antigen is the earliest indicator of ganglion cell differentiation yet reported. The existence of RA4 positive apolar cells along the outer surface of the retina suggests that the ganglion cell phenotype is expressed as soon as the cell becomes postmitotic. Approximately 20% of the migrating ganglion cells were paired. One interpretation of this observation is that ganglion cells are generated by a ganglion cell stem cell that on its ninth division undergoes a terminal division. Immunoblots and other analyses revealed the RA4 antigen to be a 140kDa cytoplasmic protein in the retina. RA4 is also expressed by many long tract axons in the brain. In the brain, the RA4 antigen was observed to have 7 different molecular weights. Evidence suggests that different cell types may express the RA4 antigen with slightly different molecular weights. Finally, the expression of the RA4 antigen in retinal axons appears to be regulated by some factor produced by the optic tectum.

453.13

IN VITRO ANALYSIS OF RADIAL GLIAL DEVELOPMENT IN EMBRYONIC RAT BRAIN. M.B. Wilkie* and J.M. Lauder, Dept. of Cell Biol. and Anat., Univ. of N.C. Sch. Med., Chapel Hill, NC 27599

A simple method for culturing relatively pure populations of radial-like glial cells was devised based on differential adhesion to a non-adhesive substrate. Dissociated cells from embryonic day 13-15 rat brain regions were plated onto glass coverslips in culture medium consisting of BME + 10% NuSerum (Collaborative Res.) + pen/strep + dextrose. Due to the initial lack of substrate adhesion, reaggregates were formed which subsequently attached and spread. Regional differences were observed in the size of aggregates and their ability to spread on the substrate, suggesting distinct cell surface properties. Most neurons did not survive after 1-2 days and the remaining cells consisted mainly of undifferentiated neuroepithelial cells or glia as determined by immunoreactivity (IR) with anti-GFAP (Accurate Chem). GFAP-IR cells initially had a radial-like appearance with long processes extending as spokes out of the aggregates. After several days, nuclei were visible within these processes, apparently moving through them to their distal ends. These cells then extended lamellipodia and retracted their proximal process which had been attached to the aggregate, forming a free-roaming GFAP-IR cell strongly resembling an astrocyte. This culture system may prove useful for investigations of the radial glial lineage.

453.14

CELL MIGRATION AFTER OLFACTORY PLACODE TRANSPLANTATION IN XENOPUS. H. Kim, A.G. Monti Graziadei and P.P.C. Graziadei. Dept. of Biological Sciences, Florida State Univ., Tallahassee, FL 32306-3050.

From stage 23-24 Xenopus laevis we transplanted the olfactory placode to same stage Xenopus borealis in place of the optic vesicle. Differential nuclear staining with quinacrine of the two animals allowed to distinguish the cells of the host from those of the donor. Chimerae were observed up to stage 52. Usually the transplanted placode fused with the one of the host producing a very large olfactory organs. In some animals the donor placode grew independently from the host's. From the transplanted placode a nerve developed and reached the diencephalon. Along this nerve many cell with the characteristics of the donor migrated and penetrated the CNS. After stage 45 these cells formed small aggregates. Attempts are under way to determine the specific nature of the migrating cells (glia versus neurons) and their significance and putative connections in the new environment.
Supported by NSF grant CNS 8617022.

453.15

NOTCH IS REQUIRED FOR CELL DECISIONS IN THE *DROSOPHILA* RETINA. Ross L. Cagan* and Donald F. Ready[1]. Dept. of Biology, Princeton University, Princeton, N.J. 08544, and [1]Dept. of Biol. Sci., Purdue University, West Lafayette, In. 47907.

We have examined the role of the gene *Notch* during successive stages in the developing retina of *Drosophila melanogaster*. Using flies containing the temperature-sensitive allele N^{ts1}, we shifted larvae and pupae to a non-permissive temperature for brief periods and examined the resulting defects. A diversity of abnormalities were observed, including transformation of one cell type to another. *Notch* appears to play a role in each retinal cell's choice of fate.

Shifting N^{ts1} larvae to 32°C resulted in normally uncommitted cells becoming photoreceptor cells; more posterior ommatidia showed differences in the number of cone cells. Shifts of young N^{ts1} pupae affected the number of bristles formed, while later shifts affected pigment cells. Each cell type was affected if shifts occurred at the approximate time it would normally differentiate. These changes in cell fate were at the expense of uncommitted cells and were not a result of new cell divisions. Late temperature shifts phenocopied *facet-glossy*, an allele of *Notch*. Our studies show the cells which would normally become primary pigment cells in *facet-glossy* flies develop instead as apparent secondary pigment cells. Together these defects and others suggest *Notch* has a role in allowing cells in the retina to receive information from neighboring cells, and that this information is required by cells to attain their correct fate.

453.16

DEVELOPMENT OF CELL-SPECIFIC MARKERS IN CHICK SPINAL GANGLION NEURONS. C.L. Smith, Dept of Neurobiology, Anatomy, and Cell Science, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

During development of the dorsal root ganglia, neural crest cells differentiate to form a variety of distinct types of sensory neurons that differ in their sizes, connections, and histochemical properties. I am investigating the role of peripheral targets in determining the phenotypes of sensory neurons by studying the expression of cell-specific markers by neurons in chick spinal ganglia. Four markers that label subsets of sensory neurons in mature embryos have been studied: an antibody to a calcium binding protein (CBP), a monoclonal antibody raised against chick ganglion cells (7G4; see Frank, et al., this volume), anti-substance P (SP), and soybean agglutinin (SA). The sensory neurons that express these markers are concentrated in different regions of the ganglia and differ in average size. Some neurons are both SA^+ and CBP^+ or SA^+ and $7G4^+$ but few neurons are both $7G4^+$ and CBP^+ or both $7G4^+$ and SP^+. Immunocytochemical studies in conjunction with retrograde labeling have shown that CBP^+ neurons are predominantly cutaneous afferents. 7G4 and SA label both cutaneous afferents and muscle afferents.

Some sensory neurons express 7G4 or SP immunoreactivity at E5 when few neurons have innervated their peripheral targets, suggesting that the early expression of 7G4 and SP is not target-dependent. Furthermore, sensory neurons from E5 embryos express 7G4 immunoreactivity when grown in vitro in the absence of their targets. By contrast, CBP and SA positive neurons are not observed until E10 or E12, after many sensory neurons have already innervated their targets. Sensory neurons from E5-E10 embryos do not become CBP^+ when grown in vitro for 4-6 days. Experiments are now in progress to determine whether sensory neurons grown in vitro become SA^+ or SP^+ and whether neurons can be induced to express specific markers by growing them with peripheral targets.
Supported by grant NS24470 to C.L.S. and NS24373 to E.Frank.

453.17

A MONOCLONAL ANTIBODY THAT SELECTIVELY LABELS LARGE SENSORY NEURONS IN DEVELOPING CHICK SPINAL GANGLIA. E. Frank, H. Tsuruhara*, C.L. Smith and V. Lemmon, Dept of Neurobiology, Anatomy, and Cell Science, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Studies of the development of various classes of sensory neurons within dorsal root ganglia (DRGs) would be greatly facilitated by the availability of histological markers for the different neuronal phenotypes. By generating monoclonal antibodies against lumbar DRGs in chick embryos, we have isolated one such marker, 7G4. This antibody stains approximately 50% of neuronal somata in E17 lumbar DRGs. Very few of the small DRG cells that are anti-substance P-positive stain with 7G4, while many of the larger neurons that label with anti-N-acetylaspartylglutamate (anti-NAAG, see Kowalski et al., Brain Res. 406:397) are also 7G4-positive. Axons in the dorsal and ventral roots and in the dorsal columns of the spinal cord are also labeled with 7G4. Bundles of stained axons descend from the dorsal columns through the dorsal horn to terminate in the intermediate laminae, similar to the projections of muscle sensory afferents. Although the staining pattern is qualitatively similar to that seen with RT97, which labels neurofilaments, 7G4 stains fewer axons in the spinal cord and stains some motoneuronal somata. Developmentally, sensory axons are 7G4-positive by E5, but staining of DRG neuronal somata does not appear until E10 and the number of labeled somata increases until at least E15. At E12, there is a clear division between large ventrolateral (VL) and small dorsomedial (DM) DRG cells, and 7G4 labels neurons primarily in the VL region. At later stages some 7G4-positive neurons are found throughout the DRG. The relative number of immunoreactive cells is 4-5 times greater in lumbar versus thoracic DRGs; this may be related to the smaller amount of cell death of the VL population at limb versus thoracic levels. Finally, 7G4 labels both muscle and cutaneous sensory neurons. Neurons in the mesencephalic nucleus of V, known to project largely to muscle spindles, are 7G4-positive, as are many large neurons in the opthalmic division of the trigeminal ganglion, which project primarily to skin. Similarly, DRG neurons retrogradely labeled with fluorescent latex beads from limb muscles are 7G4-positive, as are axons in cutaneous nerves in the hindlimb.
Supported by NS24373 to E.F.

453.18

DETERMINATION OF A NEURONAL LINEAGE BY EARLY CLEAVAGE STAGES. B.C. Gallagher & S.A. Moody. Depts. Anatomy & Neuroscience, Univ. Virginia, Charlottesville, VA 22908.

In order to examine whether early interactions are necessary for the normal production of a single frog blastomere's progeny, we transplanted single labeled cells of the 16- and 32-cell stages to novel sites in unlabeled hosts. Embryos were fixed at late tailbud stages, serially sectioned, and the distribution of the labeled cells compared to normal fate maps (Moody, 1987, Dev. Biol. 119:560; 122:300). Some transplanted blastomeres took on the characteristics of their new site. For example, V1.1.2 normally is a significant contributor of Rohon-Beard neurons, but becomes a significant contributor of motoneurons after transplantation to the dorsal midline. Some blastomeres, however, continued to express their normal neuronal and non-neuronal lineages after transplantation. For example, the D1.1 clone produced after transplantation to the ventral vegetal pole was qualitatively indistinguishable from normal. In about a third of these latter experiments, and in all experiments in which V1.2 was transplanted to the ventral vegetal pole, secondary neural tubes were induced. The D1.1 clone was normally distributed in this secondary axis. Supported by Grants NS23158, NS07868, and NBBD Training Grant HD07323.

453.19

BLASTOMERE INTERACTIONS ARE NECESSARY FOR CNS LINEAGE EXPRESSION BY SOME FROG CELLS. S.A. Moody & D.W. Best. Depts. Anatomy & Neuroscience, Univ. Virginia Sch. Med., Charlottesville, VA 22908.

We investigated whether interactions between cleavage stage blastomeres are necessary for the production of their normal neuronal progeny. Single blastomeres in the third tier of the 32-cell stage (vegetal equatorial cells) were marked with a lineage dye and their anterior neighbor was removed. The dorsal midline cell normally contributes sparsely to the hindbrain and spinal cord, but after ablation it no longer contributed at all. The dorsal lateral cell normally contributes many cells to brain and spinal cord; after ablation it did not contribute to CNS in half of the embryos and contributed only a few cells in the rest. The ventral lateral cell normally contributes to dorsal hindbrain and spinal cord; after ablation its only neuronal progeny were a few Rohon-Beard neurons. The ventral midline cell normally contributes sparsely to dorsal spinal cord; after ablation it had no neuronal descendants in half of the embryos, and had the normal ones in half of them. The non-neuronal progeny of these cells were not altered by the ablations. Thus, interactions between second and third tier cells specifically influence CNS lineage expression in the frog. Supported by NS23158.

453.20

SOME NEURONAL LINEAGES ARE ALTERED BY LITHIUM TREATMENT DURING FROG CLEAVAGE STAGES. S.L.Klein and S.A.Moody. Dept. of Anatomy and Cell Biol. Univ. Virginia Sch. Med., Charlottesville, VA 22908.

Previous studies have shown that Xenopus embryos that are incubated in LiCl during cleavage stages become dorsalized and produce up to twice the normal number of neurons (Breckenridge et al., Devel., 99:353, 1987). With our methods, the critical period of Li^+-mediated dorsalization is between the 32- and 512-cell stage. We investigated whether the increase in neuronal number was caused by changes in neuronal lineages. The fates of individual blastomeres of 16-cell Li^+-treated embryos were compared to their fates in the normal embryo (Moody, Dev. Biol., 119:560, 1987). A ventral animal blastomere (V1.1), whose normal neuronal descendants are few and located only in the spinal cord, populated large amounts the forebrain, midbrain, hindbrain and spinal cord. A ventral vegetal blastomere (V2.1) and a dorsal animal blastomere (D1.1) produced significantly more progeny in regions of CNS that they normally populate. A dorsal vegetal blastomere (D2.1) produced small amounts of the CNS, as in the normal embryo. These results indicate that neuronal lineages normally are influenced by events that occur during cleavage stages and that these events are altered by Li^+ ions. Supported by HD23324 & NS23158.

NEURAL PLASTICITY IN ADULT ANIMALS: ANATOMY AND BEHAVIOR

454.1

ANATOMICAL AND BEHAVIORAL EVIDENCE FOR A TIME DEPENDENT VULNERABILITY TO LESION 2 OF 2-STAGE BILATERAL SENSORIMOTOR CORTEX LESIONS IN RATS. T.A. Jones*, T.M. Barth and T. Schallert, Dept. Psychology and Institute for Neurological Sciences, Univ. Texas, Austin, 78712

Previous research in this laboratory has shown that there may be a sensitive period following brain damage during which recovery processes are vulnerable to pharmacological manipulations (Hernandez et al., 1988). In the present study, we found that there may be a sensitive period in recovery following unilateral forelimb sensorimotor cortex (FLSMTR) lesions during which the contralateral homotopic cortex is especially vulnerable to damage. Rats were given 2-stage bilateral FLSMTR lesions with interoperative periods (IOPs) between 1 and 14 days. To compare the extent of damage incurred by Lesion 1 to Lesion 2, the volume of the intact cortex contiguous to each lesion was measured. Two-stage lesions with a 7 day IOP showed greater damage (less remaining cortical volume) in the 2nd lesioned hemisphere. However, the volume of the Lesion 1 cortex did not differ from the Lesion 2 cortex when 2-stage lesions were given with 1 or 14 day IOPs. Furthermore, sensorimotor behavioral asymmetries, as measured by a bilateral tactile stimulation test, paralleled the anatomical asymmetries. The lateralized behavior following 2-stage lesions with a 7 day IOP and the smaller cortical volume in the Lesion 2 hemisphere indicate that the cortex contiguous to the 2nd lesion may be anatomically vulnerable to damage at this stage in recovery. In other words, more extensive damage occurs to the cortex when previous damage was given in the opposite homotopic cortex. However, there appears to be a fixed stage or time period before and after which this vulnerability does not exist. Supported by NIH grant NS-23964 awarded to T.Schallert.

454.2

COMPARISONS OF INFANT AND ADULT LESIONS ON VISUOSPATIAL BEHAVIOUR IN RATS. D. Christie* and A. Cowey* (SPON: European Brain and Behaviour Society). Dept. of Experimental Psychology, University of Oxford, South Parks Road, Oxford. OX1 3UD. U.K.

Lesions of anteromedial cortex in rat produce a neglect for visual stimuli in the field contralateral to the lesion. Recovery from the gross symptoms of the neglect occurs after 2-3 weeks but subtle effects persist. It is widely believed that when lesions are made at birth there is greater recovery, or sparing, than when they are made in adult animals. This may be a reflection of the greater degree of neuroanatomical plasticity demonstrated in the neonate. The degree to which this occurs following lesions in either the anteromedial or striate cortex was examined. Unilateral lesions of anteromedial or visual cortex were made at birth (postnatal day 1) or at three months. All animals were given a three month recovery period then visuospatial behaviour was examined. The results demonstrate that the degree of recovery depends on the task as well as the age at operation. In a 2-choice spatial task (T-maze) adult-operated animals showed a bias towards the ipsilateral arm. Neonatally-operated animals did not. In an open field, containing an array of objects, all animals with lesions of anteromedial cortex directed their search behaviour initially towards the ipsilateral side of the apparatus. In contrast rats with neonatal lesion of visual cortex showed a contralateral preference whilst adult-operated animals did not show a bias. The results also suggested that the nature of the turning behaviour depended on the hemisphere damaged. Damage to the left hemisphere produced ipsilateral turning behaviour whilst identical damage to the right hemisphere caused the animals to turn contralaterally. This asymmetry was especially noticeable in the animals with lesions of anteromedial cortex.

454.3

AMPHETAMINE ENHANCES BEHAVIORAL RECOVERY FROM SENSORY-MOTOR DEFICIT RESULTING FROM INFARCTION OF PRIMARY SOMATOSENSORY CORTEX. B.E. Hurwitz, W.D. Dietrich, P.M. McCabe, B.D. Watson, M.D. Ginsberg & N. Schneiderman. Depts. of Psychology, Neurology and Cerebral Vascular Research Center., Univer. of Miami, Coral Gables, FL 33124.

The present study was undertaken to determine whether d-amphetamine (d-AMP) would differentially affect the rate of recovery of task performance after unilateral whisker cortical barrel-field infarction relative to control groups. Food-deprived adult male Wistar rats were trained in the dark to produce a right turn in a T-maze apparatus when the vibrissae on the right side of the animals' snout were deflected by a manually delivered probe. Unilateral (n=14) or sham (n=8) infarction of the left primary whisker cortical barrel-field was induced after an 80% level of correct responses was achieved during three successive sessions. Post-infarction testing was performed every other day from the third to the 35th day. All animals were tested on post-infarction day 3 to establish whether a behavioral deficit was present. Infarcted animals displayed a significant 24.3 ± 5.4 (SEM) decrease in percent correct response. Then in these infarcted animals d-AMP at 4mg/kg (n=5), d-AMP at 2mg/kg (n=5) or saline (n=4) injections were given about 24 hrs prior to testing on post-infarction days 4, 6, 9 and 11. Of the eight sham-control animals five received no injections and three received injections of d-AMP at 2mg/kg. No effect of the sham-operation or of d-AMP was observed on task performance in these control animals. However, all three infarct groups displayed a significant increasing linear trend in percentage of correct responses between post-infarction day 3 and 35. Most notably, amphetamine-enhanced recovery of performance was observed in the 4 mg/kg group, which significantly differed from the infarct-saline group from day 17 to 35. These data, provide further evidence of the facilitatory effect of d-AMP on behavioral recovery from brain injury and extend this effect to the recovery from infarction of the primary somatosensory cortex. Supported by research grants NHLBI (5 T32 HL07426-09) & USPHS (NS-05820).

454.4

ACCELERATED RECOVERY FROM LEARNED ALTERNATION DEFICITS AFTER PROGRESSIVE ENTORHINAL CORTEX LESIONS IN RATS. J.J. Ramirez*, M. Valbuena*, M. Smith* and S.T. Wallenius* (SPON: B. Fass). Dept. of Psychology, Davidson College, Davidson, NC 28036.

Sprouting by the crossed temporodentate pathway (CTD) has been implicated in the recovery of spatial alternation behavior after unilateral entorhinal cortex (EC) lesions in rats. Presumably, if sprouting contributes to behavioral recovery, systematic manipulations of the rate of sprouting should produce concomitant changes in behavior. Previously, Scheff et al. (1977) demonstrated that 2-stage (progressive) EC lesions accelerate sprouting in the dentate gyrus of rats. In the present study, we wanted to determine whether progressive unilateral EC lesions would accelerate the recovery from lesion-induced alternation deficits. Rats that sustained 1-stage or progressive lesions were tested for retention of a spatial alternation task. Whereas rats with 1-stage lesions recovered to preoperative performance levels 10 days postlesion, the progressive lesion group recovered as early as 4 days postlesion. Histological analyses indicated that the lesions of both groups were comparable. Therefore, progressive lesions accelerated behavioral recovery. This finding is consistent with the possibility that CTD sprouting contributes to recovery from alternation deficits. However, further anatomical studies are required to confirm the presumptive accelerated CTD sprouting. Supported by NIH grant 1 R15 NS24948-01.

454.5

SYNAPTIC REORGANIZATION IN THE HIPPOCAMPUS LEADS TO CHANGES IN ENERGY METABOLIZING ENZYMES. I.L. Wagman* and R.C. Collins. Dept. of Neurology, UCLA Sch. of Med., Los Angeles, CA 90024.

In the hippocampus, terminal zones of the perforant pathway from the entorhinal cortex show high levels of cytochrome oxidase (CO), whereas terminal zones of the commissural and associational fibers show high levels of lactate dehydrogenase (LDH). To study the role of afferent pathways on postsynaptic levels of energy metabolizing enzymes, we examined the distributions of CO and LDH in the dentate gyrus following lesions of the entorhinal cortex in rats. Animals received unilateral electrolytic lesions of the entorhinal cortex. After survival periods of 8 or 16 hrs. (n=1 per time point) or 1, 2, 4, 8, 16 or 30 days (n=3 per time point), serial cryostat sections were prepared and stained for cell bodies (thionin), degenerating nerve terminals (Fink-Heimer), acetylcholinesterase, or CO or LDH activity. Enzymatic changes were analyzed with densitometry. At 16 hrs. postlesion, CO staining in the deafferented outer molecular layer had decreased to 78% of contralateral control values. Levels fell to 70% of control by 8 days with no further changes up to 30 days. LDH reactivity was 88% relative to the contralateral side at 24 hrs. postlesion, decreased to 77% by 8 days and remained about the same at 30 days. A relative increase of 12-17% was observed in the inner molecular layer of the deafferented side at 24 hrs. postlesion for both CO and LDH, returning to control values by 4 days. In addition, an expansion of the band of intense staining for LDH in the inner molecular layer was observed at 8 days postlesion. At 30 days, the LDH band was 140% of its normal width. This expansion parallels that of the commissural-associational fibers following entorhinal cortex lesions. These results may reflect control of oxidative and glycolytic enzyme levels by pathway-specific synaptic input.

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454.6

EARLY PLASTICITY IN THE DENTATE GYRUS FOLLOWING SURGICAL LESIONS TO THE PERFORANT PATH. D. E. Hillman and S. Chen. Dept. Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016.

The number of synaptic sites in the dentate molecular layer begins to be restored by 5 days and returns to near normal by 60 days following lesions to afferent pathways. The source of the new sites may be from sprouting of the ipsi- and contralateral hippocampi and the septal nuclei. A surgical lesion was placed in the entorhinal cortex of the rat at the junction with the subiculum. Morphometry of the heads of spines and synaptic sites at 5 days revealed elongation of the spines so that they partially wrapped the bouton. The average size of the synaptic contact area increased by as much as 40% and additional sites occurred on the extension. Three-dimensional reconstruction revealed larger sites, irregularly shaped contact areas, and multiple sites on the same spine. We conclude that increases in the size of synaptic sites precede restoration of synaptic number following acute deafferentation of the hippocampus. These findings indicate that a significant number of the restored sites could arise from remaining synapses through dividing of the contacts and their respective spines. Supported by USPHS NS-20349 & NS-13742 from NINCDS.

454.7

Entorhinal cortical lesions are accompanied by an alteration in the expression of a ganglioside in the dentate molecular layer. S.F. Suchy, J.J. Ramirez*, M. Valbuena* and G.A. Schwarting*. Dept. of Biochemistry, E.K. Shriver Center, Waltham, MA 02154 and Dept. of Psychology, Davidson College, Davidson, NC 28036.

A ganglioside, α-galactosyl, α -fucosyl GM1, which is present in low quantities in rat brain is recognized by a monoclonal antibody, WCC4 (Suchy et al., Brain Res. 440,1988). This ganglioside is concentrated in the outer 2/3 of the molecular layer (ML) of the dentate gyrus. WCC4 antibody was used to detect changes in the distribution of α-galactosyl, α-fucosyl GM1 in the dentate ML following unilateral lesions of the entorhinal cortex (EC). Nine 90-day old male Sprague Dawley rats were administered unilateral electrolytic lesions of the EC and were sacrificied at 3 or 7 days postlesion. Immunocytochemical analysis of fresh frozen brain was performed with WCC4 antibody. The width of the immunostained portion of the ML, expressed as a fraction of the total ML width, was determined for the deafferented (ipsilateral) and contralateral ML from photographs of tissue sections. To account for time dependent "shrinkage" of the denervated ML following EC lesions, a ratio of the proportion of the immunostained ML ipsilateral/contralateral (I/C) to the lesion was calculated for each animal. At 3 days, there was no difference in immunostaining between ipsilateral and contralateral ML (I/C = 1.01 ± 0.13, n = 5). However, at 7 days postlesion there was a significant ($p<0.01$) reduction in staining in the ipsilateral EC (I/C = 0.67±0.09, n = 4). It is apparent that there is a redistribution of, or loss of, the antigen in the outer 2/3 of the molecular layer following EC lesions. The change in α-galactosyl, α-fucosyl GM1 expression in the ML corresponds to the time of expansion of commissural/associational terminal field (Lynch et al., Brain Res. 50, 1973) and the degeneration of dendrites in the deafferented zone (Caceres and Steward, J. Comp. Neurol. 214,1983). Supported by NS2494-01.

454.8

PLASTICITY IN THE CROSSED AND UNCROSSED NIGROSTRIATAL PROJECTIONS IN RELATION TO RECOVERY FROM BEHAVIORAL ASYMMETRIES INDUCED BY HEMIVIBRISSOTOMY. J.P.Huston, H.-T. Weiler*, S.Morgan* and H.Steiner*. Inst. Physiological Psychology, Univ. Düsseldorf, D-4000 Düsseldorf, F.R.G.

Unilateral removal of vibrissae (URV) in rats induces an asymmetry in facial scanning in the open-field. This behavioral asymmetry subsides after 3 days of URV. Evidence exists for plastic changes in the crossed nigrostriatal projection subsequent to URV. In this experiment we determined the time-course of this neural plasticity and compared it to that of the behavioral recovery. After URV for 1-3, 4-6, or 8-20 days rats were injected with horseradish peroxidase into the striatum (CPU) ipsilateral or contralateral to URV. Retrogradely labeled cells were counted in both substantiae nigrae. URV/1-3 rats had a greater crossed projection to the CPU ipsilateral than contralateral to URV. This asymmetry was reversed in URV/4-6 rats, and reduced in URV/8-20 rats. The latter group had a greater uncrossed projection to the CPU contralateral to URV. Thus, these results indicate a similar time-course for the neural plasticity as for the behavioral recovery after URV.

Supported by DFG-grant Hu 306/3-3.

454.9

RAPID FORMATION OF NEW DOUBLE SYNAPSES IN RAT SUPRAOPTIC NUCLEUS (SON) IN RESPONSE TO INTERRUPTION OF SUBFORNICAL ORGAN EFFERENT PROJECTIONS. M.L. Weiss, C.D. Tweedle, F. Marzban*, B.K. Modney*, and G.I. Hatton. Michigan State Univeristy, Neuroscience Program, East Lansing, MI 48824-1117

Previous work at the transmission electron microscopic (TEM) level has shown that damage of the subfornical organ (SFO) produces degenerating terminals within the SON (Brain Res. 275: 365, 1983). In an attempt to replicate and extend this study, rats received damage to the SFO or its efferent pathway (SFOx) and, following a survival period of 24 hr, were prepared for TEM evaluation. In addition to finding occasional degenerating fibers, we found an increase in the percentage of cells contacted by terminals which formed synapses upon two adjacent somata in the SON (soma-somatic "double" synapses, SSD) after SFOx [mean of 41% cells with SSD after SFOx, range: 3-8 cells with double synapses per 15 cells sampled per animal (N = 5) vs. mean of 0% cells contacted by SSD after sham surgery, 0 cells with SSD per 15 cells sampled per animal (N = 3), and following sham surgery and 24 hr water deprivation: mean of 2% cells contacted by SSD, range 0-2 cells contacted by SSD in 15 cells sampled per animal, (N=3)]. It is interesting to note that the large increase in the percentage of cells contacted by SSDs after SFOx was accompanied by little increase in cell-cell apposition and that the synapses formed were morphologically similar to those found during lactation or chronic dehydration. Supported by NS 09140 and NS 16942 and NRSA Postdoctoral award 08125.

454.10

DEHYDRATION INDUCED INCREASES IN RAT SUPRAOPTIC NEURONAL CELL SIZE IS ACCOMPANIED BY ABSOLUTE INCREASES IN MULTIPLE SYNAPSES, NEURONAL MEMBRANE APPOSITION AND GLIAL CONTACT. B. K. Modney*& G. I. Hatton. (SPON: A.K. Salm) Neuroscience Program, Department of Psychology, Michigan State University, East Lansing, MI 48824.

Extensive alterations in rat SON morphology occur during chronic dehydration. Comparisons made between well-hydrated (n=6) and dehydrated rats (10 days drinking 2% saline, n=7) revealed significantly increased percentages of cell membrane contacted by adjacent cells or dendrites and multiple synapses (a single terminal forming two synaptic contacts). A significant decrease in the percentage of somatic membrane contacted by single synapses was found. No change in percentage contacted by glial processes was found. Since the neuronal surface area (SA) of these cells increases significantly with dehydration, the percentage measures listed above were multiplied by SA to determine the μm^2 per cell contacted by each cellular element. No change in the amount of somatic membrane contacted by single synapses was found. In contrast, glial contact, somatic/dendritic membrane apposition and multiple synapses were found to increase in dehydrates relative to controls. Direct membrane apposition and the formation of new double synapses may play an important role in the coordinated activity of these neurons during periods of chronic hormone release. Supported by NIH grant NS09140.

454.11

METABOLIC PLASTICITY IN THE HIPPOCAMPUS FOLLOWING SYMPATHETIC SPROUTING V. S. Yip Dept. of Anatomy, Univ. of Anatomy, Salt Lake City, UT 84132.

The invasion of the hippocampus by sprouts of peripheral sympathetic fibers following medial septal lesions provides a unique model for studying collateral sprouting in the CNS. The sympathetic sprouts are mainly restricted to the hilus and granule cell and supragranular layers of the dentate gyrus, and to the stratum lucidum and stratum radiatum of CA3 of the hippocampal formation (HF) where they replaced the lesioned septohippocampal neurons (Crutcher, K.A., Brothers, L. and Davis, J.N., Exp. Neurol., 66:778, 1979). The aim of the present experiment is to study the biochemical changes associated with the cellular events which takes place when, as the result of a medial septal lesion (loss of cholinergic fibers), the HF is invaded by noradrenergic sympathetic fibers from the superior cervical ganglion. The medial septums of rats are electrolytically lesioned under anesthesia, the brains are processed by Nissl, cholinergic and adrenergic histochemistry and quantitative microchemical analysis of two key oxidative enzymes of the citrate cycle: malate dehydrogenase (MDH) and citrate synthase (CS). The Lowry microchemical methods are used for precise localization (confirmed by histochemistry) and quantification of MDH and CS activities in the appropriate layers of the septally denervated HF. At 2 weeks after lesion, the sympathetic sprouts can be seen in the granule cell and supragranular layers of the dentate gyrus and the MDH and CS activities in the sprouted areas were depressed compare to control levels whereas the enzyme activities in the outer molecular of the HF and Layer IV of the cortex where no sprouting occurred remained unchanged. At 4 weeks post lesion, the MDH and CS levels were still low but to a lesser degree compare to the control levels. The results suggest the MDH and CS activities of sprouted sympathetic fibers are metabolically different than septohippocampal fibers and are specifically localized in the sprouted areas only.

454.12

SEROTONERGIC INNERVATION OF THE SUPERFICIAL LAMINAE OF RAT SUBNUCLEUS CAUDALIS (SpVc) FOLLOWING ADULT INFRAORBITAL (IO) NERVE TRANSECTION. B.G. Klein. Dept. of Neurosci., New York Coll. of Osteo. Med., Old Westbury, N.Y. 11568.

Serotonin immunoreactive (5-HTIR) axons which project to SpVc of the normal rat are highly concentrated in lamina I & outer lamina II, forming a dense, longitudinally oriented band. The possibility that 5-HTIR axons show a reactive alteration of this distribution following disruption of normal primary afferent input to SpVc was investigated in 4 adult rats, more than 60 days after unilateral IO nerve cut. In the IO region of SpVc, on both the normal & nerve cut sides, the superficial & deep borders of laminae I & II, as well as the dense band of 5-HTIR axons, were outlined at 113X. There was no significant change in the average width of the superficial laminae following adult IO nerve cut (normal, $\bar{X}$=98.3±15.1 u; nerve cut, $\bar{X}$=106.5±20.9 u). Mean % area of laminae I & II occupied by the dense band of 5-HTIR axons also did not change significantly (normal, $\bar{X}$=58.3%±4.7%; nerve cut, $\bar{X}$=57.7%±2.7%). The mean density of 5-HTIR varicosities (var) within the superficial laminae of the IO region of SpVc was estimated at 1111X. Again, no significant difference was observed following IO nerve cut (normal, $\bar{X}$=82599±10659 var/mm^2; nerve cut, $\bar{X}$=80887±9599 var/mm^2). Thus, adult IO nerve cut does not alter the area or terminal density of the heavy band of 5-HTIR axons within laminae I & II of the IO region of SpVc. Support:DE07734.

454.13

REINNERVATION OF SUPRAOPTIC NUCLEUS AFTER VENTRAL NORADRENERGIC/MEDIAL FOREBRAIN BUNDLE LESIONS: SOURCE OF NEW AFFERENTS. C. J. Phelps and S. W. Carlson*. Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

Fluorescence histochemical and physiological evidence for reinnervation of SON vasopressin-secreting neurons, after long-term neurosurgical lesion of noradrenergic afferents to the nucleus, has been reported (Phelps & Sladek, Brain Res. Bull. 13: 727, 1984). The source of this reinnervation is being studied 1) by retrograde tract-tracing methods, and 2) by eliminating contribution of peripheral vascular sympathetic sprouting by superior cervical ganglionectomy (SCGx). In intact or sham-operated rats, retrograde transport of wheat germ aggulutinin coupled to horseradish peroxidase (WGA-HRP) injected into SON consistently labelled cells in ventrolateral medulla (A1). At 4-5 days following mechanical mfb transection, retrograde transport of WGA-HRP failed to label perikarya in A1. Fluorescence microscopy showed significant loss of catecholaminergic (CA) afferents to SON after mfb "deafferentiation," but not after SCGx alone. By 14 days after combined mfb lesion + SCGx *vs.* mfb lesion alone, fluorescent innervation of SON, only in animals with intact SCG, indicated significant contribution by peripheral vascular CA afferents, although simultaneous regeneration at the lesion site was observed. Current experiments are testing the morphological significance of SCG *vs.* A1 contributions to SON after long-term deafferentation. Supported by NIH grant AG 06139 (CJP).

454.14

EVIDENCE THAT RUBROSPINAL NEURONS SURVIVE AXOTOMY IN THE ADULT OPOSSUM. G.F. Martin and X.M. Xu. Department of Anatomy and Neuroscience Program, The Ohio State University College of Medicine, Columbus, Ohio 43210.

As part of ongoing studies on rubrospinal plasticity in the opossum, we sought to determine if rubrospinal neurons survive axotomy in the adult animal. Fast-Blue (FB) was injected into the thoracic cord of 3 anesthetized animals and 4 days later they were reanesthetized and the rubrospinal tract cut 4 segments rostral to the lesion. Approximately 30 days after the lesion, the animals were anesthetized again and sacrificed by perfusion so that frozen sections of their spinal cord and brain could be examined with a fluorescence microscope. In each case, neurons were labeled in the red nucleus contralateral to the lesion. In one case, FB spread to both sides of the cord making it possible to compare the size and location of labeled rubrospinal neurons contralateral and ipsilateral to the lesion. In that case, labeled neurons on the contralateral side were smaller than those on the ipsilateral side. In 2 other animals, the rubrospinal tract was lesioned at the same level and after about 1 month the red nucleus was injected with wheatgerm agglutinin conjugated to horseradish peroxidase. Labeled axons could be traced to the lesion site where they appeared swollen, but not beyond it. We interpret our results to show that rubrospinal neurons survive axotomy for at least 1 month but their axons do not grow caudal to the lesion. (Supported by NS-25095).

454.15

RESPONSE TO SUBICULAR STIMULATION IN LAMINAE II/III AND V OF RAT CORTICAL SLICE. T.G. Hedberg Dept. Physiology Boston Univ. Sch. Med., 80 E. Concord St. Boston, MA 02118

In associative learning paradigms, subicular inflow is integral to development of neural population responses in cingulate cortex which may differ markedly between laminae. In the rat, synaptic inflow from subiculum reaches area 29c via a fiber tract with terminal field largely in lamina II/III. To investigate the response properties of pyramids in laminae II/III and V, paracoronal slices of rat cingulate cortex containing this fiber tract (SCT) were prepared. Subiculum was stimulated with square pulses lasting 0.2 msec. Intracellular responses obtained from 23/23 lamina V impalements were pure EPSPs driven at a $\bar{x}$ latency of 3.6 msec which supported 1-2 APs. In contrast, 2/27 lamina II/III impalements were not driven, 6/27 were driven with pure EPSPs, 14/27 with mixed EPSP/IPSPs and 5/27 with pure IPSPs. Lamina II/III PSP onset was >6.0 msec and EPS EPSPs and mixed EPSP/IPSPs supported 1-5 APs. Increases in stimulus amplitude (>500 uV) or frequency (>0.2 PPS) resulted in changes in PSP shape and latency indicating monosynaptic excitatory drive on lamina V neurons and polysynaptic mixed-sign drive on lamina II/III neurons.

Thus, a model SCT activation of area 29c involves: 1) monosynaptic excitation of local circuit interneurons and deep pyramids via their apical tufts, 2) polysynaptic activation of superficial units via ascending pyramidal collaterals, 3) feedback regulation of deep pyramids.

454.16

DIFFERENTIAL LAMINAR DISTRIBUTION OF ASTROCYTIC PROCESSES IN THE HIPPOCAMPAL DENTATE GYRUS. A. Marks*, K. R. Isaacs, A. M. Sirevaag, F. -L. Chang, W. T. Greenough. (Spon.:B. Oakley). Neural & Behav. Bio. Prog., Depts. of Psych and Cell & Struct. Biol. U. of Illinois, Champaign IL, 61820.

To our knowledge, no one has reported a quantitative analysis of the distribution of glial processes in the dentate gyrus of the rat. As prelude to investigating the role of glia in plasticity, it was necessary to have a reliable estimate of the pattern of glial processes. 8um frozen sections were taken from the septal end of the hippocampus of young adult male rats (60-70 days old) and labelled with antibody to GFAP, an astrocyte specific marker. Astrocyte ramifications were quantified using the method of Braendgaard and Gundersen (1986), and provided a stereologically unbiased estimate of astrocyte surface area (Sv) in the molecular layer of the dentate gyrus. The dentate gyrus molecular layer is usually divided into three approximately equal subdivisions corresponding to the inputs of the lateral and medial perforant path and the commissural /associational axons and we used this division as a template for collecting our data. A significant difference in the astrocytic Sv (in um^{-1}) was found in all 3 layers: Outer layer=72.90, Middle layer=57.70, Inner layer=41.84 ($p < 0.0001$). There were no significant differences between upper and lower blades. (Supported by NIMH, ONR and EFA).

454.17

RATS REARED IN A COMPLEX ENVIRONMENT HAVE LARGER ASTROCYTES WITH MORE PROCESSES THAN RATS RAISED SOCIALLY OR INDIVIDUALLY. A.M. Sirevaag, S. Smith, and W.T. Greenough. Depts. Psych. & Cell & Struct. Biol., & Neur. & Beh. Biol. Prog., Univ. IL, Champaign, IL 61820.

Rats raised in complex environments (EC) have larger neurons with more synapses in the occipital cortex than rats raised socially (SC) or in individual cages (IC) (e.g. Turner & Greenough, Brain Res., 1985). These results suggested that synaptic communication might be more intense in ECs than ICs. Astrocytes have been shown to be involved in ionic and neurotransmitter regulation and so may also influence the efficacy of synaptic transmission. Preliminary examination of astrocytes in the cortex of EC, SC and IC rats showed that astrocyte nuclei were larger in ECs than ICs. This implied that astrocytic arborization might also be greater in ECs than ICs. This hypothesis was tested by using indirect immunocytochemistry to label astrocytic processes with anti-GFAP and then estimating the surface area (Sv) of those processes by the stereological technique of vertical sections. This study shows that ECs have a 17% greater Sv of astrocytic process than ICs; that the density of astrocytes is 12% lower in ECs than ICs; and that the mean surface area per astrocyte is 27% greater in ECs than ICs. SCs values are intermediate on all measures. Supported by PHS 2T32GM07143 and MH 35321.

454.18

4 DAYS OF DIFFERENTIAL HOUSING ALTERS DENDRITIC MORPHOLOGY OF WEANLING RATS V.L.Kilman*, C.S.Wallace, G.S. Withers & W.T. Greenough. Neur. & Beh. Biol. Prog., Depts. Psych. & Cell & Struct. Biol., Univ. IL, Champaign, 61820.

Previous studies have demonstrated that 30 days of housing in a complex environment alters dendritic morphology of various neuron types in rat visual cortex. This study assessed these changes after a much briefer exposure to complex housing. Weanling rats were used, as exploration activity peaks at this age. 12 littermate pairs of 30 day old male Long-Evans hooded rats were randomly assigned to differential housing for 4 days. Complex housing for one littermate consisted of a large cage containing other rats and an assortment of toys (EC). EC rats spent one hour per day in a play cage while toys in the home cage were replaced. Littermates of the EC animals were housed individually (IC) for 4 days. Basilar dendrites of layer III pyramidal cells were drawn from Golgi impregnated sections. Total dendritic length and number and length of branches at each order of bifurcation were analyzed. EC rats exceeded IC rats in total dendritic length. These results indicate that the effects of exposure to a complex environment are manifest rapidly in weanling rats and are compatible with the hypothesis suggested by previous results (Turner & Greenough, Brain Research., 1985) that the effects of a complex environment on neuronal morphology involve synaptogenesis in response to environmental stimulation. Supported by NIMH 35321

454.19

COMPLEX EXPERIENCE INDUCES CAPILLARIES IN VISUAL CORTEX OF ADULT RATS. J.E.Black, A.M.Zelazny*, & W.T.Greenough, College of Medicine, Depts of Psychology and Cell & Structural Biology, and Neural & Behavioral Biology Program, University of Illinois, Urbana 61801.

Young rats reared with other rats and toys generate many new capillary branches within 30 days (Black, etal, Neurosci Lett, 83: 351), but angiogenesis in old rats provided complex experience seems to be impaired (Isaacs, etal, Soc Neur, 12: 1579). The present study examines angiogenesis in 66 3-month-old rats either given complex experience (EC) or kept in isolation (IC) for periods of 10, 30, or 60 days and then perfused. All vessels are easily seen in toluidine-stained 1 μm sections of visual cortex. The visual cortex of EC rats was significantly thicker than that of IC rats after 10, 30, and 60 days. This corresponds to previous estimates of greater synaptogenesis in EC rats (Hwang & Greenough, Soc Neur, 12: 1284) which presumably spread apart existing blood vessels. Capillary spacing was nearly identical for EC and IC rats after 10 and 30 days, however, indicating that EC rats made new capillaries. EC rats also had a smaller mean capillary diameter than IC rats after 10 days, consistent with the presence of many immature and small capillaries. These findings suggest that angiogensis in the mature cerebral cortex begins within 10 days of complex experience and requires at least 60 days to reach completion. Supported by NIMH.

BEHAVIORAL PHARMACOLOGY: ACETYLCHOLINE

455.1

EFFECTS OF SCOPOLAMINE ON A DELAYED SPATIAL MATCHING TASK IN RHESUS MONKEYS. R.F. Genovese and T.F. Elsmore*. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

We investigated the effects of scopolamine (1-32 µg/kg) on a novel spatial learning task where three rhesus monkeys were trained to interact with images on a CRT. Eight circles, arranged in a circular pattern, were presented on the screen and one circle (chosen randomly) contained a distinctive feature. Touching the featured stimulus cleared the display for .01, 4, or 16 s. A choice screen consisting of the eight circles, without the featured stimulus, was then presented. Touching the circle that had previously contained the feature, produced a food pellet and was considered a correct choice.

Under baseline conditions, accuracy was approximately 95%, 85%, and 60% for the .01-, 4-, and 16-s delay conditions, respectively. Scopolamine produced dose-dependent decreases in the number of trials completed in all three monkeys. Only small decreases in accuracy were observed at the .01-s delay. In some instances, much larger decreases in accuracy were observed at the 4- and 16-s delays. In all cases, doses of scopolamine producing decreases in accuracy also produced substantial decreases in the number of trials completed.

These results extend previous studies of cholinergic involvement in spatial learning tasks in rodents and further, suggests that the procedure may be useful for assessing the effects of a variety of compounds in primates.

455.2

SCOPOLAMNE INDUCED VISUAL MEMORY DEFICITS IN RHESUS MONKEYS. N.L. Yusuf, S.F. Sands and D.E. Moss. Departments of Neurology and Neurophysiology, University of Texas Health Science Center, 6431 Fannin, Houston, Texas 77225.

This study investigated the effects of scopolamine, a muscarinic receptor agonist, on a visual recognition task in two adult male rhesus monkeys. The monkeys were initially trained to perform on a serial probe recognition (SPR) task with a variable list length design that closely parallels tests given to humans: a simultaneous discrimination and a 1-,2-,4- and 8-item multiple retention task. A dose-dependant decline in baseline performance was observed following intramuscular injections of varying concentrations of scopolamine HBr (.003mg/kg - .025mg/kg). The scopolamine administration resulted in a significant decrease in performance on the longer list lengths indicating impaired memory function. Performance on the simultaneous discrimination, which does not involve a memory component, showed no significant change from control days. The decline in visual recognition ability in these monkeys closely resembles the decline in memory observed in humans with cholinergic insufficiencies, i.e., Alzheimer,s disease. This data suggests a strong cholinergic component in this type of visual retention task. This paradigm provides an excellent opportunity to explore new and current therapeutic approaches in memory disorders by enhancement of cholinergic transmission.

455.3

EFFECTS OF DAILY REPEATED SOMAN EXPOSURE ON TRACKING PERFORMANCE AND BLOOD CHOLINESTERASE IN RHESUS MONKEYS. D. W. Blick*†, M. R. Murphy†, G. C. Brown*†, S. Z. Kerenyi*†, & S. L. Hartgraves†. *Systems Research Laboratories, & †Radiation Sci. Div., USAF School of Aerospace Med., Brooks AFB, TX 78235-5301.

We estimated the daily dose of soman, an organophosphate that irreversibly inactivates cholinesterase (ChE), required to produce a small but reliably detectable decrement in the performance by rhesus monkeys of a well-learned compensatory tracking task, the Primate Equilibrium Platform (PEP) task. Monkeys were tested sequentially at soman dosages (0.84 - 1.06 µg/kg/day) set by the up-and-down method. The threshold dosage (ED50) for a performance decrement on or before the 5th daily exposure was found to be 0.97 µg/kg/day (95% confidence interval: 0.80 $\leq$ ED50 $\leq$ 1.18 µg/kg/day), about 40% of the single-dose acute ED50. Behavioral effects of daily repeated soman exposures are much more variable than the effects of a single, acute exposure. Blood ChE inhibition is a poor predictor of behavioral effects.

Tests for interactions between chronic ChE inhibition by pyridostigmine and performance effects of daily repeated soman exposure are in progress.

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455.4

ANTICHOLINERGIC REVERSAL OF HALOPERIDOL'S DELETERIOUS EFFECTS ON FORELIMB STEADINESS AND RESPONSE INITIATION IN RATS. S.C. Fowler, R.M. Liao, P.D.Skjoldager, C. Bass, and W.D. Brown. Depts. of Psychology and Pharmacology, University of Mississippi, University, MS 38677

Rats were trained to reach through a rectangular hole and exert downward forces on a force transducer. As long as the force on the transducer was maintained above 20 g the rats were able to drink from a dipper of sweetened condensed milk. After a two-week period of 10-min daily practice on this task, the rats were divided into 4 groups and treated with ip. injections of vehicle (V) or haloperidol (Hal, 0.04, 0.08, 0.16 mg/kg) for 17 consecutive days. On the 16th drug day each of the 4 groups received an ip. injection of atropine sulface (AS, 5mg/kg) in addition to Hal. The dependent variables were time-on-task (the amount of time the rat's forelimb force remained above 20 g) and the integrated variance ("power") of force oscillations having frequencies within a 5-to-25 hertz bandwith. Hal alone reduced time-on-task and increased the variance of force oscillations. When AS was given along with Hal, the disruptive effects of Hal on performance were significantly attenuated. These data suggest that the behavioral deficits engendered by low doses of haloperidol in rats are analogous to neuroleptic-induced parkinsonian symptoms in human beings. (Supported, in part, by MH 43429.

455.5

GLUCOSE ATTENUATES DRUG-INDUCED HYPERACTIVITY IN MICE: EFFECTS ON CENTRAL CHOLINERGIC FUNCTION. D.L. WALKER*, W.S. STONE, R.J. RUDD*, and P.E. GOLD. Dept. Psychol., Univ. Virginia, Charlottesville VA 22903.

Glucose (GLU) attenuates scopolamine (SCOP)-induced amnesia and hyperactivity, as well as atropine-induced changes in paradoxical sleep. Additionally, GLU augments the severity of physostigmine (PHYSO)-induced tremors. This study addresses the specificity of GLU interactions with cholinergic function and examines whether such effects are mediated by central or peripheral GLU actions.

Locomotor activity of mice was assessed by counting lines crossed during 10 min in a 52.5 cm^2 enclosure. 45 min prior to testing, animals were injected (IP) with saline (SAL), morphine-sulphate (MS; 10 mg/kg), or amphetamine (AMPH; 1 mg/kg). 15 min prior to test, animals received SAL, GLU (10, 50, 100, or 500 mg/kg) or PHYSO (0.01, 0.05, 0.1, or 0.2 mg/kg). GLU and PHYSO blocked MS- but not AMPH-induced hyperactivity. Because hyperactivity induced by opiates but not AMPH may be mediated by inhibition of cholinergic function, these results are consistent with a specific interaction of GLU with cholinergic systems. A second group of mice received SCOP (3 mg/kg) 45 min and fructose 15 min before testing. Fructose, which is not utilized by the CNS, did not attenuate SCOP-induced hyperactivity, suggesting that the locomotor effects of GLU are mediated centrally. (Supported by MH 31141, ONR N00014-85-KO472, the American Diabetes Association, and training grant MH18411).

455.6

CHOLINERGIC-DOPAMINERGIC INTERACTIONS IN RADIAL-ARM MAZE PERFORMANCE: ROLE OF NICOTINIC CHOLINERGIC SYSTEMS. S.R. McGurk, E.D. Levin, and L.L. Butcher. Department of Psychology, University of California, Los Angeles, CA 90024-1563.

Interference with either muscarinic-cholinergic or dopaminergic function impairs radial-arm maze performance. Paradoxically, the amnestic effect of muscarinic antagonism is significantly reduced by simultaneous dopaminergic blockade, suggesting that cognitive function is depedent upon a balance between these neurotransmitter systems. Since central nicotinic receptors are involved in cognitive functioning, and because blockade of nicotinic receptors impairs maze performance, this experiment examined the interaction of nicotinic-cholinergic and dopaminergic systems.

Rats were trained on an 8-arm radial maze. Drug testing began after asymptotic levels of performance were reached. All rats received saline, mecamylamine (2.5, 10.0 mg/kg), haloperidol (0.04, 0.08 mg/kg), or a combination of mecamylamine and haloperidol (2.5 and 0.04 mg/kg, respectively) in a counterbalanced order. Maze performance was significantly impaired by the higher doses of mecamylamine or haloperidol. Interestingly, although the lower doses of mecamylamine and haloperidol did not affect maze performance, their combination caused a significant decrease in performance.

Taken together, these results reinforce the importance of dopaminergic and nicotinic-cholinergic systems in maze performance and indicate a significant interaction of these systems. That subthreshold doses of nicotinic-cholinergic and dopaminergic antagonists summate to interfere with maze performance suggests that the circuitry underlying this behavior contains both a nicotinic and dopaminergic synapse. Finally, these data illustrate the differential involvement of muscarinic and nicotinic systems in learning. (NIH grant NS-10928 to L.L.B.).

455.7

NICOTINE ENHANCES PLAYFUL ATTACK IN THE PLAY-FIGHTING BY JUVENILE RATS. S.M. Pellis, V.C. Pellis and R.L. Lennon* Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Systemic (S.C.) injection of nicotinic acid produced a dose dependent increase in play-fighting in juvenile rats (30-40 days old). By using 'pinning' (ie. one animal standing over the supine partner) as the measure of play-fighting the increase was not significant over vehicle controls at 0.125 mg/kg but was so at 0.5 mg/kg. However, pinning involves both attack and defense, so that an increase in pinning could arise from an increase in either attack rate or defense rate. Therefore, attack (ie., lunge at nape) and defense (ie. rotate to supine, withdrawing nape from attacker) were measured separately for each member of each pair.

After 24h isolation sibling rats were reunited, 15 min after one animal received an appropriate injection and their behavior was videotaped under red-light for 10 min. Attack in the injected rats increased significantly with increasing dose, whereas defense remained unchanged. Neither the rate of attack or defense increased in the non-injected partners. These results show that nicotinic stimulation facilitates only one of the two components involved in play-fighting, so that overall increases in play-fighting are produced by elevated frequencies of attack, not of defense.

455.8

CONSEQUENCE OF NICOTINE TREATMENT ON A SPATIAL TEST OF MEMORY. S.A. Welner, A. Nair*, A. McNicoll*, P. Boksa and R. Quirion. Douglas Hosp. Res. Ctr., McGill Univ., Dept. of Psychiatry, Montreal, Quebec, H4H 1R53.

The consequence of a schedule of nicotine treatment to rats that is said to increase the specific binding of [^{3}H]ACh or [^{3}H]nicotine to nicotinic cholinergic sites in the cerebral cortex was tested on the acquisition and memory of platform location in a Morris water maze. Rats were treated for 5 days with saline or a low dose (0.2 mg/kg s.c.) of nicotine administered following each day's trials. Previous reports that this treatment increases locomotor activity in the first minutes following injection were confirmed. Nicotine treatment did not alter consolidation of the spatial task because the rate of task acquisition, measured as latency to find the platform, was not different in the two groups. After 2 days without treatment, at a time when nicotinic receptor up-regulation in the cortex is said still to be present, the animals were retested for their memory of the task and again, no difference was observed between the two groups. In an attempt to augment cholinergic function, tetrahydroaminoacridine (THA) at a dose of 5 mg/kg was administered 1 hr pre-test to all animals; a significant decrease in latency to find the platform from the first trial to the last trial was observed in the group treated with nicotine. (Supported by MRC Canada).

455.9

RATS DEVELOP A PREFERENCE FOR NICOTINE SOLUTIONS. F.W. Flynn and C. Ksir. Dept. of Psychology and Neuroscience Program, University of Wyoming, Laramie, WY 82071

The responses of rats to nicotine solutions were examined using the taste reactivity (TR) test and 2-bottle 24 hr preference test. In Experiment 1, non-deprived rats were administered intraoral infusions of water, 1 µg/ml, 5 µg/ml, 10 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml nicotine, and TR responses were videotaped and analyzed. Nicotine, up to 50 µg/ml, elicited ingestive TR responses. Ingestive responses significantly decreased and aversive TR responses significantly increased in response to 100 µg/ml nicotine. Next, 2-bottle preferences for water vs. 1 µg/ml, 5 µg/ml and 0 µg/ml (water control group) were measured in 3 groups of naive rats. After 16 days of exposure, rats showed a significant preference for 1 µg/ml nicotine. The preference for 5 µg/ml nicotine significantly increased during the experiment, but it remained less than that for 1 µg/ml and 0 µg/ml nicotine. Last, TR responses elicited by 1 µg/ml and 5µg/ml nicotine were measured in the rats having had the 2-bottle experience. Rats showing a 2-bottle preference for the 1µg/ml nicotine showed significantly more ingestive TR responses to 1 µg/ml and 5 µg/ml nicotine that did the control rats. These data show that prolonged voluntary access to nicotine results in an increased preference for nicotine and modifies the rats' immediate oral/gustatory reactivity to nicotine.
(Supported by NS-24879 awarded to F.W.F.)

455.10

NICOTINE EFFECT ON LOCOMOTOR ACTIVITY IS NOT REDUCED BY DOPAMINE RECEPTOR BLOCKADE E. J. Cline and C. Ksir. Department of Psychology, University of Wyoming, Laramie, WY 82071.

Nicotine increases locomotor activity in rats, especially after several days of treatment. Evidence suggests a role for the mesolimbic dopamine system in this effect. Clarke et al (Soc Neurosci Abstr 13:406, 1987) demonstrated that nicotine increased locomotor activity and dopamine turnover in the nucleus accumbens and olfactory tubercule. Ksir and Cline (Soc Neurosci Abstr 13:447, 1987) showed that 6-OHDA lesions of the nucleus accumbens reduced nicotine-induced locomotor activity.

Rats were given daily s.c. injections of 0.2 mg/kg nicotine for seven days prior to behavioral testing. They were habituated to the test cages for one hour and then pretreated with either saline, 0.05, 0.1, or 0.2 mg/kg alpha-flupenthixol (FPT), a dopamine receptor antagonist, and returned to the test cages for 90 minutes. Rats were then tested for one hour following a dose of 0.2 mg/kg nicotine. Tests were repeated on subsequent days so that all rats received each pretreatment. Pretreatment with FPT did not reduce the number of cage crossings during the 20 min following nicotine administration.

One week later the same rats were tested for their response to 1.0 mg/kg d-amphetamine after pretreatment with either saline or 0.2 mg/kg FPT. FPT pretreatment decreased the locomotor response to d-amphetamine ($F=16.15$, $df=1,12$, $p<0.01$).

It is puzzling that blocking postsynaptic dopamine receptors does not interfere with the behavioral response to nicotine.

455.11

MAPPING OF THE EFFECT OF CARBACHOL ON LOCOMOTION IN THE BASAL FOREBRAIN OF THE RAT. S.M. Brudzynski, R.S. McLachlan and J.P. Girvin. Epilepsy Res. Lab., Dept. of Clin. Neurol. Sci., University Hospital, London, ON N6A 5A5 Canada.

Intracerebral injection of carbachol into the basal forebrain is known to decrease spontaneous locomotor activity of rats. The anatomical location of this pharmacological response is not precisely known. The goal of the present study was to quantitatively map the response to carbachol in the basal forebrain and anterior diencephalic areas. Intracerebral injections of carbachol were performed in 48 animals (total 91 brain sites) in a dose of 1 µg (5.47 nanomole - the dose close to D_{50}) and in a volume of 200 nL into different sites between the frontal planes 6.5 and 9.5 mm from the interaural plane. Locomotion, expressed as a percent of the saline control, was measured by the total distance covered by an animal and the total time of movement within 10 min after injection using an automated activity monitor. The strongest and the most consistent decrease of locomotion occurred from the medial preoptic and the anterior hypothalamic areas, particularly medial preoptic nucleus, in contrast to ventromedial hypothalamic nucleus, retrochiasmatic area, paraventricular nucleus and diagonal band where increase in locomotion was found.

Supported by the Ontario Mental Health Foundation and the PSI Foundation.

INTERHEMISPHERIC RELATIONS

456.1

THE SIZE OF THE HUMAN CORPUS CALLOSUM RELATES TO LANGUAGE LATERALIZATION AND TO VERBAL ABILITY. M. Hines[1], K. Sloan[1*], J. Lawrence[1*], J. Lipcamon[2*] and L. Chiu[2*]. [1]Dept. of Anatomy and Neuropsychiatric Institute, UCLA School of Medicine, Los Angeles, CA. 90024-1763. [2]Dept. of Radiology, Harbor/UCLA Medical Ctr., Torrance, CA. 90509.

The size of the corpus callosum and some of its subregions have been reported to vary with sex and handedness. In this study we examined relationships between callosal size and cognitive functions that vary with sex or handedness. Subjects (29 women, 20-45 years of age) completed measures of verbal fluency, visuospatial ability and language lateralization and had magnetic resonance images (MRIs) made of their brains. The midsagittal MRI was used to measure the cross-sectional surface area of the callosum and its subregions, including the posterior fifth (P5) and the posterior third minus fifth (P3-5), which have been reported to vary with sex and handedness, respectively. Verbal fluency related positively to callosal area, particularly to P5. Language lateralization related negatively to callosal area, particularly to P5 and P3-5. Visuospatial ability did not relate to callosal measures. These results suggest that individual differences in language lateralization and in verbal ability may depend on the number of the size of fibers in the corpus callosum and its subregions. (Supported by funds from the UCLA Neuropsychiatric Institute and by USPHS NS20687 to MH.)

456.2

A SEX DIFFERENCE IN THE STRUCTURAL ASYMMETRY OF THE CAUDATE NUCLEUS USING MRI SCANS IN HUMANS. Richard S. Lewis[1], Stephanie B. Hodson[1*], Jim Lipcamon[2*], Lee Chiu [2*] & Melissa Hines[2]. 1. Department of Psychology, Pomona College, Claremont, CA. 91711; 2. UCLA School of Medicine.

The present study investigated an anatomical asymmetry, and its variation with sex, in the volume of the left and right caudate nucleus.

Twenty six normal right-handed adults (13 males and 13 females) received inversion recovery magnetic resonance imaging (MRI) scans. The volume of the head of the caudate nucleus was traced throughout the horizontal and coronal sections and the volume of the caudate was calculated using the Bioquant Software system and an IBM PC. A laterality index was derived, (R - L)/(R + L) * 100, from the average volume values of the horizontal and coronal sections.

A significant sex difference in asymmetry was observed, $t(24) = 2.02$, $p=.055$, such that males tended to have a larger right than left caudate nucleus and females a larger left than right caudate nucleus.

456.3

STRIATE AND EXTRASTRIATE ASYMMETRIES IN JUVENILE AND ADULT HUMAN BRAINS. M-C de Lacoste, C-N Kim*, N. Smith*, & D. J. Woodward, Dept. Cell Bio., U. T. Southwestern Medical Ctr., Dallas, TX 75235

We have previously reported striking occipital regional volumetric asymmetries in a large number of primate species at all phylogenetic levels (*de Lacoste* et al., 1988). The aim of this study was to determine if, in juvenile and adult human cerebra (n=8), this "gross" asymmetry reflects an underlying asymmetry in primary visual (striate,[ST]), extrastriate [XT] , or in both ST and XT cortex.

Series of 35-50 μm Nissl and myelin stained coronal sections, obtained from both the *Yakovlev* collection and our laboratory, were videodigitized. The CSCAN utility of CARP (Computer Aided Reconstruction Package) was used for semi-automated delineations of 1) the external and internal borders of cortical gray and 2) the boundaries between ST and XT (per techniques described in [*Smith, de Lacoste* et al 1987]). CARP was also used to compute regional areas and volumes.

Preliminary results indicate that both XT and ST are highly asymmetrical in adult humans (XT: 7-122%; ST: 11-30%). In fact, occipital regional volumetric asymmetries based on cytoarchitecturally defined regions are two- to sixfold more pronounced than those we quantitated at the gross level. We believe that the reason for this is that ST and XT asymmetries favor opposite hemispheres. Our data further suggest that juvenile and adult males exhibit greater ST and XT asymmetries than their female counterparts. In sum, our results indicate that both primary and association visual areas are asymmetrically organized in the left and right hemispheres of the human brain. Future studies will assess volumetric asymmetries in subdivisions of XT i.e. visual association cortex. *Supported by HD21711 to MCL and the Biological Humanics Foundation.*

456.4

MORPHOMETRY AND OPTICAL DENSITOMETRY OF THE CORPUS CALLOSUM IN MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA: A MAGNETIC RESONANCE IMAGING STUDY. R.D. Sanders*, M. F. Casanova*, T. Goldberg*, L. Bigelow*, G. Christison*, D. R. Weinberger and E. F. Torrey* (SPON: A. P. Oliver). Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032

Many of the symptoms, cognitive deficits and evoked potential abnormalities of schizophrenia (SC) may be due to aberrant interhemispheric communication. To test this hypothesis, several planimetric magnetic resonance imaging (MRI) and postmortem studies have analyzed the structure of the corpus callosum (CC). Results thus far have been contradictory. In the present study, we have used a computer image analysis system (LOATS) in combination with MRI's to examine the morphology and optical density of the CC in 12 pairs of monozygotic twins (5 female, 7 male) discordant for SC. No differences in CC area (anterior, middle, posterior thirds and total), length, vertical height of the CC body (at three levels) or optical density (at four points) were demonstrated by t-test comparisons of SC and their normal cotwins. Quantitative harmonic shape analyses showed no significant differences between normal and SC cotwins, but suggest effects of gender on posterior CC shape (1st harmonic $p = 0.007$, 3rd harmonic $p = 0.049$; Kruskall-Wallis), and a gender-diagnosis interaction effect on anterior CC shape ($p = 0.043$; MANOVA). These results fail to replicate prior findings of altered length, thickness and area in the schizophrenic CC, but may partially support gender-related differences in the shape of the splenium.

456.5

APPENDING SYMBOLIC INFORMATION TO GRAPHICAL REPRESENTATIONS OF NEUROANATOMICAL DATA: APPLICATIONS IN VISUAL CORTEX. W.K. Smith, N.C. Smith*, M.C. de Lacoste, D.S. Schlusselberg and D.J. Woodward. *Dept. of Cell Biology and Anatomy and Dept. of Physiology, U. Texas Southwestern Med. Ctr., Dallas, TX 75235.*

Ongoing studies in this laboratory have explored the application of computer graphics and image processing techniques to three-dimensional reconstruction from serial sections of neuroanatomical tissue. The CARP system (Computer Aided Reconstruction Package) contains software modules which allow manual tracing, morphometry, computer microscopy, C-14 2DG autoradiography analysis, three-dimensional reconstruction and image synthesis. A sophisticated hierarchical, anatomical database provides storage for varied forms of data including video images, graphical representations of tissue sections, and three-dimensional surface models. Experience has revealed a need for the ability to attach arbitrary text descriptions to graphical and image data which represent associations between the stored data and information from other sources. For example, boundaries denoting cortical regions may have appended multiple anatomical names, descriptors or lists of bibliographic references. A screen-oriented menu allows users to manipulate a LISP data structure called the *property list* which associates property-value pairs with a node in the CARP database. Once created, the property lists serve both as cross references with other information sources and as targets for searching paradigms in CARP database operations. Current applications include assigning complex descriptions to subregions of striate and extrastriate visual cortex in primate brain. *Support from DA2338, AA3901 and the Biological Humanics Foundation to DJW, HD21711 to MCL and SBIR NS25321 to WKS (Biographics, Inc.).*

456.6

HEMISPHERIC ASYMMETRIES IN LOGICAL CATEGORIES: CONVERGENT EVIDENCE FROM NORMAL AND COMMISSUROTOMY SUBJECTS. D. W. Zaidel and K. Frederick. Dept. of Psychology, UCLA, Los Angeles, Ca 90024.

In a previous study (D.W. Zaidel, Cognitive Neuropsych., 4:321-332, 1987), hemispheric asymmetry was found for retrieval of information about natural categories from long-term semantic memory. The purpose of the present study was to determine whether any asymmetries are present for logical categories. Both normal and patients with complete section of the forebrain commissures were studied. The task consisted of deciding whether or not numerals are ODD or EVEN. Stimulus numerals represented the following bins: S=<10, M=>10<20, or H=>40<50. They were flashed in the left or right visual half-fields and the answer was indicated with a key-press. Both accuracy and latency was recorded. Results showed a high accuracy rate in both visual fields. Analysis of latencies revealed significant interactions for field X parity and for field X bin (e.g.,S,M,or H). The latency results are interpreted to reflect hemispheric asymmetries in processing logical categories, where inclusion is based on necessary and sufficient conditions. This extends previous observations of hemispheric asymmetries in retrieval from long-term semantic memory for natural categoies and supports the assumption of specialized storage/retrieval even in the presence of the forebrain commissures.

456.7

HEMISPHERIC PROCESSING OF GRATINGS IN A COMMISSUROTOMY PATIENT. Robert Fendrich* and Michael Gazzaniga, Div. of Cognitive Neuroscience, Cornell Univ. Med. College, N.Y., N.Y., 10021

To test the hypothesis that the left hemisphere in humans is specialized for the processing of high spatial frequency information, while the right hemisphere is specialized for the processing of low frequency information, pairs of sinusoidal gratings were presented within the left and right visual fields of a commissurotomy patient. The gratings employed had spatial frequencies of 1, 2, 4, or 8 c/^{o}, and either horizontal or vertical orientations. The gratings were windowed by a circular Gaussian approximately 1.5^{o} across, and had a mean luminance of 31 cd/m^{2} and a maximum contrast of 66%. The two gratings in each pair were vertically aligned and flashed to the subject's left or right visual field 1.5^{o} from the vertical midline. Presentations lasted 167 msec. and were terminated by a composite grating mask. The two gratings in each pair were identical in spatial frequency but could differ in orientation. The subject reported if their orientations were the same or different.

Accuracy data provides no indication of a relative advantage for high frequencies in the RVF, or a relative advantage for low frequencies in the LVF; the data in fact shows the opposite trend. Accuracies for the 4 spatial frequencies (going from 1 to 8 c/^{o}), based on 64 trials per condition, were 81%, 83%, 77% and 63% in the RVF, and 67%, 75%, 79%, and 81% in the LVF. This suggests that at the processing levels required by the experimental task, the subject's left and right hemispheres were not specialized for the respective processing of high and low spatial frequency information.

456.8

AN INVESTIGATION OF HEMISPHERIC LATERALIZATION USING A PHOTIC PROBE PARADIGM. J.M. Rothfeld*, A.S. Zeiberg, C.W. Linebaugh, H.A. Emsellem. Depts. of Neurology and Linguistics, The George Washington Univ., Wash., DC 20037

Interhemispheric electrocortical asymmetries have been demonstrated during the processing of afferent sensory information in the human using auditory and visual evoked potential (VEP) recordings. The photic probe paradigm in conjunction with brain electrical activity mapping (BEAM, Caldwell - model 8400) of VEPs was utilized to characterize interhemispheric electrocortical responses to auditory stimuli. Pattern reversal VEPs were recorded from 20 right-handed subjects (10 males/10 females) using the standard 10-20 electrode system during inattentive and attended states to music (non-verbal) and continuous speech (verbal) stimuli.

Examination of the BEAM scans and a cross-correlation analysis generated from 8 bilateral recording sites revealed no reproducible hemispheric asymmetries associated with any of the conditions. VEPs recorded under all conditions were normal for latency and amplitude. No reproducible asymmetries were noted on separate analysis of F7-F8, T3-T4, C3-C4, O1-O2. The inability to detect previously reported electrocortical asymmetries may reflect the modality of the probe or the complex asynchronous nature of the auditory stimuli.

456.9

INDUCING EMOTION BY UNILATERAL CONTRACTION OF FACIAL MUSCLES: A LOOK AT HEMISPHERIC SPECIALIZATION AND EMOTION. B. Schiff* and M. Lamon* (Spon: I. Zucker). Dept. of Psychology, University of Toronto, Toronto, Ont. M5S 1A1.

This research investigated whether hemispheric function in emotion could be studied by arousing each hemisphere through unilateral contractions of contralateral facial muscles in the lower third of the face. We discovered that sustained contractions of the left facial muscles induced sadness. Right facial muscle contractions abolished the dysphoria produced by the left face contraction and produced a consistent but difficult to characterize mixture of positive affect and aggression. This was replicated in an objective study in which judges reliably identified the laterality of the facial contraction from transcripts of the subjects' reports. In a third experiment the performance of these facial contractions had comparable effects on the emotional tone of stories told about an ambiguous picture. These results indicate that emotions can be turned on by sustained unilateral facial muscle contractions. They are consistent with the view that the right hemisphere is implicated in negative emotional experiences and that the left hemisphere has a different but not easily specified function. They provide a method for studying hemispheric specialization for emotional experience and have implications for relations between emotion and cognition.

456.10

COGNITIVE AFFECTIVE CHANGES FOLLOWING RIGHT OR LEFT TEMPORAL LOBECTOMY FOR EPILEPSY. A.K. Collings*, R.F. Stevens*, and L. Switzman, Dept. of Psychology, Wellesley Hospital, Toronto, Canada, M4Y 1J3.

Clinical observations of epileptic patients in hospital for temporal lobectomy suggest that those with a clear and isolated L temporal epileptogenic focus have disrupted social interactions on the ward more often than those with a R focus. Consequently, we undertook to quantify this with 10 measures of cognitive-affective functioning pre- and post-surgically. Significant trends were found in 4 measures, most notable being a significant interaction in Social Imperturbability. This was higher pre-surgically in the L patients, than in the R patients, with post-surgery reduction (i.e., improved social sensitivity and awareness) in the L patients only. This variable has usually been associated with dysfunction of the frontal lobe (Lezak, 1983) not the temporal lobe. We are currently correlating this neurobehavioral finding with indices of R and L frontal and temporal cognitive dysfunction to clarify potential connections between L temporal epileptic activity and disruption of frontal lobe functions.

456.11

DICHOTIC LISTENING IN RELATION TO SEVERITY OF CLOSED HEAD INJURY. H.S. Levin, W.M. High*, D.H. Williams*, E.G. Amparo*, and H.M. Eisenberg. The University of Texas Medical Branch, Galveston, TX 77550

Eighty-two right-handed patients who sustained a closed head injury (CHI) of varying severity were characterized according to the level of the deepest lesion visualized by magnetic resonance imaging (MRI). Patients with no lesions or extradural and/or cortical lesions were grouped together (n=31) while patients with lesions extending into the subcortical white matter or deeper comprised another group (n=51). Thirteen right-handed controls were also examined. All patients and controls were screened for hearing loss and tested on dichotic listening using a computer synthesized tape which consisted of six consonant-vowel nonsense syllable pairs presented simultaneously via earphones. The subjects were instructed to repeat the syllables presented on each trial. A laterality index reflected the preference for reporting the syllables heard by the left or right ears. Nonparametric analysis of variance indicated that patients sustaining moderate to severe CHI showed a significantly greater right ear advantage in dichotic listening performance than controls. Furthermore, among the moderate to severe injuries, patients with parenchymal lesions extending into the subcortical white matter showed a greater right ear advantage as compared to controls, whereas the laterality index of patients with lesions which were higher on the neuroaxis did not differ from results in controls.

456.12

LATERALITY IN MONKEYS DISCRIMINATING INVERTED FACES. B. A. Vermeire and C. R. Hamilton. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The right hemisphere of human beings is usually found to be superior to the left in the recognition of facial identity and expression. We have shown that the right hemisphere of rhesus monkeys is superior in learning, remembering, and generalizing discriminations of monkey faces. In humans, faces are relatively more difficult to recognize when inverted than are other stimuli usually seen upright, such as houses or cars; the right hemisphere seems particularly sensitive to facial inversion. In the present experiment, 16 split-brain monkeys who had previously learned to discriminate upright faces were tested with the same discriminations inverted. While the right hemisphere was superior in discriminating the upright faces ($p<.02$), there was no significant laterality for discriminating the inverted faces. Furthermore, there was a significant difference ($p<.001$) between the laterality found for upright and inverted faces. While the interpretation of the inversion deficit is controversial, the finding that the data from monkeys is similar to that from people suggests that similar mechanisms of laterality are being examined in both species.

Supported by MH-34770.

456.13

ANATOMICAL BRAIN ASYMMETRIES IN MONKEYS. Peter L. Heilbroner and Ralph L. Holloway. Department of Anthropology, Columbia University, New York, NY 10027.

Measurements evaluating hemispheric asymmetry were taken on the cortex of formalin-fixed brain specimens from two Old World and three New World monkey species. We found significantly greater mean length ($p<0.05$; n=20-30) for the left Sylvian fissure (SF) than for the right SF in all five of these samples. In accord with this finding, results of several behavioral studies (e.g. Petersen et al., Science 202:324,1978) suggest left-temporal-lobe dominance for auditory discrimination functions in monkeys. SF asymmetry is also characteristic in the human brain. However, other measurements on the temporoparietal area which usually suggest asymmetry in Homo brains did not reveal side differences in these monkey brain samples. Furthermore, asymmetry in the development of sulci in the cortical region homologous to Broca's area, in visual association cortices, or asymmetry in the surface area of the cingulate gyrus was not revealed. Evidence for functional lateralization involving these cortical regions in monkeys has not been reported.

456.14

ASYMMETRIES IN NEGLECT REINSTATED BY SPIROPERIDOL IN RATS RECOVERED FROM LEFT VS. RIGHT DORSOMEDIAL PREFRONTAL CORTEX LESIONS. J.M. Vargo*, M. Richard-Smith* and J.V. Corwin (SPON: B. King). Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148.

Dopamine (DA) agonists produce acute recovery from neglect produced by unilateral destruction of medial precentral prefrontal cortex (PCm) in rats. The present study examined the role of DA mechanisms in spontaneous long-term recovery from unilateral PCm lesions.

Subjects (Ss) (male Long-Evans hooded rats) received right (RPCm) or left (LPCm) lesions or a lesion lateral to PCm (LAT). Neglect was assessed by rating the degree of head orientation to visual, auditory, or tactile stimuli. After recovery, the Ss received either 0.03, 0.05, 0.07, or 0.10 mg/kg of spiroperidol (SPIRO), or the vehicle.

SPIRO reinstated neglect dose-dependently in PCm Ss ($p<0.001$). Neglect severity also depended on the hemisphere damaged ($p=0.02$), neglect was reinstated in RPCm Ss at lower doses. As with lateralization of neglect seen postsurgery (Vargo et al., Exp. Neurol., in press), right-sided neglect was induced in both PCm groups by SPIRO. Only RPCm Ss showed bilateral dose-dependent neglect. LAT Ss were unaffected at the optimal dose of SPIRO.

The results indicate: 1) DA mechanisms may underlie spontaneous recovery from cortical neglect, and 2) these mechanisms are organized asymmetrically.

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456.15

DIFFERENT BEHAVIORS INDUCED BY STIMULATION OF THE RIGHT AND LEFT STRIATUM. M. G. Ziegler and H.Szechtman. Department of Neuroscience, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Recently, animal studies have revealed left-right hemisphere asymmetries of neurotransmitter levels, dopamine receptor binding, glucose metabolism and morphology of the corpus striatum. Using a novel paradigm, the present study reveals that activation of the right striatum has a qualitatively different effect on behavior of rats than activation of the left striatum. To produce a relatively selective activation of the right and left striatum, the model of Ungerstedt was employed, where injection of apomorphine in a rat with a 6-hydroxydopamine lesion of the substantia nigra activates, preferentially, the postsynaptic dopamine receptors on the side of the lesion. By modifying the paradigm of Pisa and Szechtman (Neurosci. Letters, 64:41,1986), we assessed whether the direction of swimming in a large circular swimming pool was influenced by edges. We report here that with activation of the right striatum, rats were attracted to the edge of a pool and swam along it in a direction that was the reverse of their preferred direction in the middle of the pool. In contrast, with activation of the left striatum, rats showed little attraction to the edge and no consistent directional preference when swimming along it. These findings suggest that the right striatum controls contralateral orientation and the left striatum controls bilateral orientation.

456.16

THE DEVELOPMENT OF CEREBRAL ASYMMETRY IN THE RAT: A THYMIDINE STUDY. G.D. Rosen, A.M. Galaburda, and G.F. Sherman. Dyslexia Neuroanatomical Laboratory, Harvard Medical School and Beth Israel Hospital, Boston, MA 02215.

Hemispheric asymmetry of neocortical architectonic areas is defined as volumetric differences between homologous areas on the two sides. This asymmetry reflects side differences in the number of neurons, rather than in cell packing density (Galaburda et al., *Cortex*, 22: 151-160, 1986; *Neuropsychol.*, 25:853-868, 1987). Side differences in cell numbers could result from asymmetries present already in the germinal zones, lateral differences in cell proliferation, side differences in cell death or architectonic reassignment. The present study sought to determine which of these developmental factors underly the observed asymmetries.

Pregnant Wistar rats were injected with [^{3}H]Thymidine at various embryonic ages and the pups were sacrificed on postnatal days 5, 10, 30, and 60. The brains were embedded in paraffin, coronally sectioned at 10μ, mounted onto slides, processed for autoradiography, and counterstained with thionin. Estimations were obtained of the number of labelled and unlabelled neurons within architectonic areas 17 and 18a, as well as the architectonic volume of these areas.

The proportion of labelled to unlabelled neurons did not differ between the hemispheres over time indicating that there were no lateral differences in cell proliferation. Analysis to distinguish side differences in initial germinal zones from cell death and/or architectonic reassignment will be reported.

This research is supported, in part, by the Charles H. Hood Foundation.

456.17

MHC TYPE AND LATERALIZATION: DEGREE OF ASYMMETRY FOR HANDEDNESS AND SWIMMING ROTATION IN *H-2* CONGENIC MICE Robert L. Collins. Jackson Laboratory, Bar Harbor, ME 04609.

H-2 types were differentially distributed in lines of mice selectively bred for degree of handedness. Whereas G0 comprised six *H-2* types, strongly lateralized G24 HI line mice were predominantly *H-2*^{d}, and weakly handed LO line mice were mainly *H-2*^{b} (R. L. Collins, G. A. Carlson & J. H. Nadeau, *Society for Neuroscience Abstracts* 11:861, 1985). To further relate MHC type to degree of asymmetry, handedness and swimming rotation were examined in two panels of *H-2* congenic mice ($n = 334$): BALB/cBy (*H-2*^{d}) and C.B6-*H-2*^{b}; and C57BL/6By (*H-2*^{b}) and B6.C-*H-2*^{d}/*a* (HW19), B6.C-*H-2*^{d}/*b* (HW41), B6.C-*H-2*^{d}/*c* (HW101).

Mice were tested first for handedness in the unbiased or 'U-world' food-reaching task and then twice later in worlds biased opposite to their handedness—'U-R-R' or 'U-L-L' (R. L. Collins, *Science* 187:181-184, 1975). Data measures across tests were expressed as paw entries consistent with original hand preference. Swimming rotation for 2 minutes in a circular water maze was observed for three days. Swim measures were summed preferred rotations/total rotations.

Handedness tests yielded partial support for the research hypothesis: (BALB > C.B6) and female (HW19, HW101) > B6. However, male B6 and B6.C mice did not show the desired pattern. For degree of swimming there was no Sex × Genotype interaction: (HW19, HW41, HW101) > B6. Albino BALB and C.B6 mice swam hardly at all. Female mice of all groups tended to swim counterclockwise. For males, the directions were balanced.

Overall results support the hypothesis that *H-2*^{d} mice are more strongly lateralized than H-2^b mice. Either the *H-2* complex itself exerts pleiotropic influence on degree of lateralization, or genes affecting lateralization reside at loci located near the *H-2* complex on Chromosome 17. Results are consistent with aspects of the Geschwind hypothesis (N. Geschwind & P. Behan, *PNAS* 79:5097-5100).

456.18

SEX AND AGE DIFFERENCES IN THE DEVELOPMENTAL PATTERN OF BEHAVIORAL LATERALITY IN RATS. B. Zimmerberg, T. Eddy* and N. Horwitz*, Dept. of Psychology, SUNY-Albany, Albany, N.Y. 12222.

Rats exhibit consistent signs of behavioral laterality and can be useful in models studying the development of cerebral lateralization. Paw preferences in a reaching task were assessed at 25 and 75 days of age. There was a significant interaction between Sex and Age. The degree of paw preference did not differ by sex among weanling age rats. Among adult rats, females had a greater degree of paw preference. In addition, females increased their degree of preference with age, while males decreased their preference. In this study, subjects were chosen from one of three prenatal treatment histories: liquid diet with 35% ethanol-derived calories, pair-fed control or standard control. Prenatal undernutrition, but not prenatal alcohol exposure alone, significantly reduced the degree of paw preference. However, prenatal alcohol exposure did appear to cause a sexually dimorphic shift in a normally left-sided population bias. Morphometric analyses of hemispheric differences in hippocampal and neocortical volume are in progress. Sex and age differences in paw preferences may reflect hormonally-mediated differential growth patterns of the two hemispheres. (NIAAA AA07359)

456.19

LATERALIZED CHANGES IN 5HT$_2$ RECEPTORS AFTER FOCAL CORTICAL STROKES IN RATS. H.S. Mayberg, T.H. Moran, R.G. Robinson. Johns Hopkins Medical Institutions. Baltimore, MD 21205

PET studies in stroke patients have shown that right hemisphere lesions lead to increased ipsilateral to contralateral binding of spiperone (primarily to 5HT$_2$ receptors) while left hemisphere lesions do not. To assess whether these lateralized changes in cortical 5HT$_2$ receptors could be demonstrated in the rat, 3H-Spiperone (SP) autoradiography was performed 30 days after unilateral cortical suction lesions. Right lesions produced bilateral increases in total SP binding in frontal cortex (excluding the lesion site): 48% greater than after left lesions, and 23% greater than shams ($F=4.67$, $p=.03$). Increases in frontal binding were positively correlated with running wheel activity ($r=.79$, $p=.06$). Left lesions led to decreased SP binding in the contralateral cortex compared to both the ipsilateral cortex and to shams. The ratio of ipsilateral to contralateral binding was significantly increased in the left lesion group compared to both shams and right lesions in perirhinal (left=1.47±.35, sham=1.03±.11, right=0.84±.19; $F=10.2$, $p=.002$) and frontal cortex (left=1.27±.12, sham=0.98±.05, right=0.95±.08; $F=21.7$, $p=.0001$). These results demonstrate that there is a lateralized receptor response to focal injury in both rats and humans.

457.1

TWO-STEP MUSCLE TRANSPLANTATION TECHNIQUE TO DETERMINE THE ORIGIN OF THE DYSTROPHIC PHENOTYPE. E. Cosmos, J. Butler* and P. Cauwenbergs. Neurosciences Department, McMaster Univ. H.S.C., Hamilton, Ontario, Canada, L8N 3Z5.

During development ex ovo, avian muscles afflicted with HMD express an impaired ability to differentiate the metabolic enzymic profile characteristic of normal (N) glycolytic muscles. To determine if this ex ovo dystrophic (D) phenotype is programmed within D myogenic cells by day 2 in ovo, D somitic mesoderm was transplanted to replace N brachial somites. Due to the limited ability of embryos to hatch following somite transplantation and the fact that metabolic differentiation is completed ex ovo, a two-step procedure was necessary. Thus, the pectoralis derived from D somitic mesoderm grafted at day 2 in ovo to an N host (step 1) was then transplanted at day 16 in ovo to a newly hatched N chick (step 2). As a control, a similar two-step protocol was performed between N donors and D hosts. The degree of metabolic differentiation achieved was assessed histochemically (phosphorylase, SDH reactions) during a postoperative period of 8-16 wk ex ovo. Results indicate that the D phenotype of impaired metabolic differentiation was expressed by genotypically D muscles whereas genotypically N muscles differentiated normally. Thus, we conclude that specific D phenotypes, not overtly expressed until development ex ovo, are programmed by day 2 in ovo. Furthermore, these are uninfluenced by extramyogenic factors associated with a N environment during development in ovo and ex ovo. (MDAC, NSERC supported).

457.2

GROWTH OF AXON PROCESSES FROM TRANSPLANTED OLFACTORY NEURONS IN THE RAT BRAIN. E.E. Morrison and R.M. Costanzo. Dept. of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0551.

Transplantation of olfactory neurons has been successfully demonstrated in the rat brain (Morrison and Graziadei, Brain Research 279:241-245, 1983). Transplant tissue contains mitotically active basal cells and young developing olfactory neurons. As the young neurons mature they develop and grow dendrites and axon processes. In the present study, we have examined the extent to which growing axons penetrate and distribute within the host brain (parietal cortex). We observed olfactory axons originating in the transplant and growing towards the host tissue. These axons formed fascicles that penetrated deep into the host brain. However, they did not appear to form characteristic glomerular-like structures. The extent and distribution of olfactory axons within the host brain demonstrates the acceptance (plasticity) of olfactory tissue by non-olfactory regions of the CNS. These results provide further evidence that olfactory neurons may prove to be an important neural tissue in transplantation studies of the CNS.

Supported by Jeffress Research Grant J-122 to EEM and NIH Grant NS16741 to RMC.

457.3

LONG-TERM REGULATION OF β-ADRENORECEPTORS IN FETAL TRANSPLANTS AND DENERVATED HOST SOMATOSENSORY CORTEX. T.Erickson*, A.Dunn-Meynell, B.E.Levin. Neurology Service, VA Med. Ctr., E. Orange, NJ 07019.

Male Sprague-Dawley hosts had unilateral 6-hydroxydopamine locus coeruleus (LC) lesions and/or ipsilteral superior cervical ganglionectomies. Fetal (E15-16) posterior cortex (CTX) or cerebellum (CB) was transplanted into host somatosensory cortex ipsilateral to lesions or ganglionectomies with 3-6mo survivals. Specific β-receptor binding (2nm ^{125}I-iodopindolol by autoradiography) was 35% higher in host CTX layer IV (43.2+2.3fmol/mg protein) than the full cortex (layers I-VI; 33.4+1.4fmol/mg; $p<0.001$) and the β_1/β_2 ratio (ICI 118551 and ICI 89406 competitive binding) in layer IV (1.35+0.16) was the same as that in layers I-VI (1.35+0.09) in intact cortex. β_1/β_2 ratios in layer IV ipsilateral to LC lesions were reduced by 33% to 0.90+0.07 ($p<0.005$). Specific β-receptor binding in fetal CTX implants (37.4+2.7fmol/mg) was the same as ipsilateral host CTX but binding in CB implants (22.6+3.7fmol/mg) was 40% and 34% lower than CTX transplants and host CTX ($p<0.005$). The β_1/β_2 ratio in CTX implants (1.75+0.10; $p<0.05$) was 30% higher than, while CB implants had a similar ratio (1.00+0.22) to, host CTX. Neither specific binding nor subtype ratios in CTX or CB implants were affected by LC lesions or ganglionectomy. Therefore, development of transplant β-receptors was dependent upon tissue origin but not host innervation. This work was supported by VA Medical Research.

457.4

BEHAVIORAL ANALYSIS OF TRANSPLANTATION INTO THE LGN IN THE HOODED RAT. R.B.Wallace, T.Davis*, C.Haddad*, M.J.Thomas* and K.Williams*. Lab of Developmental Psychobiology, Univ. of Hartford, W.Hartford, Conn. 06117.

Past research has suggested transplantation of embryonic neocortical tissue into various brain regions of juvenile or adult host animals can be evaluated behaviorally(Wallace R.,Das,G.D.(1982) Brain Res. 243:133-139;Stein,D.G.,Micheal R.L.,Attella,J.,Rakowsky,H.(1985) Behav.Neur. Biol. 44:266-277). In an effort to further examine this issue, embryonic neocortical tissue was taken from 17 day old embryos and transplanted bilaterally into the region of the LGN in 4 mo. old male Long Evans Hooded rats. Animals receiving neocortical transplants (N=10) have been compared with animals receiving bilateral electrolytic lesions of the LGN (N=13) and with normal controls (N=16) on a battery of tests designed to assess both overall neurologic integrity and visual performance. All surgical procedures were carried out under appropriate surgical anesthesia. Following conclusion of behavioral testing, all animals were overdosed with sodium pentobarbital, transcardially perfused and the brains prepared for histology and qualitative microscopic evaluation. Behavioral examination indicates that significant differences exist on all assessments between the lesioned animals and each of the other two groups, with the control and transplant conditions being essentially comparable on the tasks examined. These results are interpreted in terms of a presumed role of the transplant tissue to allow for compensatory recovery within host animals.

457.5

SURVIVAL OF PYRAMIDAL NEURONS IN CORTICAL TRANSPLANTS GRAFTED INTO CAVITIES IN THE MOTOR/SENSORY CORTEX OF JUVENILE RATS. M.F. Gonzalez, K. Hisanaga*, J.E Loken*, J. E. Madl*, F.R. Sharp. Depts. of Neurology and Physiology, UCSF and V.A Medical Center, San Francisco, CA 94121; and Dept. of Vet. Biology, Univ. of Minnesota, Minn., MN 55455

Embryonic cortical grafts surviving in cavities made in motor/sensory cortex of adult rats contain nearly normal numbers of nonpyramidal, peptidergic interneurons (J. Neurosci. 7: 3002, 1987). However, very few retrogradely labeled pyramidal neurons are seen in similar transplants after WGA-HRP was injected into host thalamus (Exp. Neurol. 99: 154, 1987).

Juvenile rats received fetal frontal cortex transplants in motor/sensory cortex as previously described. A month later sections were processed for immunocytochemistry. A monoclonal Ab to α-tubulin was used to stain all neurons. A monoclonal to neuro-filament protein (NFP) selectively labeled pyramidal cortical neurons of layers III and V. The number of neurons in transplants and normal cortex was similar. All grafts had NFP reactive neurons but their number was less than in normal cortex. This number, however, was much greater than the number of pyramidal neurons that established connections with host thalamus in previous word. Some grafted pyramidal neurons were aligned in rows, but their processes were not perpendicular to the pial surface as in normal cortical laminae.

457.6

CROSS SPECIES TRANSPLANTS OF RODENT STRIATUM INTO A PRIMATE MODEL OF HUNTINGTON'S DISEASE: ANATOMICAL AND BEHAVIORAL EFFECTS. A.W. Deckel, D. Holmes*& W.H. Niemann*. Depts. of Psychia. and Animal Res. Facil., Univ. of Med. and Dent. of N.J., N.J. Med. Sch., 185 S. Orange Ave., Newark, N.J.

Extensive past research in our laboratory has examined the anatomical characteristics of fetal striatal transplants placed into the kainic-acid lesioned adult rodent striatum, and has suggested that these transplants have good therapeutic potential to reverse the lesion-induced behavioral deficits (for review, see Deckel and Robinson, Ann. N.Y. Aca. Sci.,.495, 556-580, 1987). This experiment reports on the effects of making similar grafts into the ibotenic acid-lesioned putamen of the primate Cynomolgus (Macaca fascicularis). Monkeys were pretrained on a rewarded alternation paradigm, requiring them to full extend their arm out of a narrow opening in their cage in order to receive a banana fixed on the end of an irregularly oscillating wire. Animals subsequently received stereotaxic IA lesions of the putamen (20ugms/2.0uls/5min). Following a two week period of recovery and post-lesion testing, animals were immunosuppressed with cyclosporin (15 mg/kg) and transplanted at 3 different sites of the lesioned putamen with day E15 rat donor striatum. Animals were subsequently behaviorally assessed for varying time periods post transplantation and examined by Nissl staining and dopaminergic/cholinergic autoradiography. This poster will discuss these results in detail.

457.7

EXPRESSION OF EXTRACELLULAR MATRIX GLYCOPROTEINS DURING ANGIOGENESIS IN NEURAL TRANSPLANTS. J.M. Krum and J.M. Rosenstein. Department of Anatomy, George Washington Univ. Sch. of Med., Washington, D.C. 20037.

Neural tissue transplants are useful models for the study of microvascular proliferation in the CNS. Transplants of both autonomic ganglia and fetal cerebral cortex are revascularized by anastomoses of host and graft vessels. The extracellular matrix molecules fibronectin (FN) and laminin (LN) form a migratory substratum for vascular endothelium in vitro; FN fragments in combination with heparin act as chemoattractants. To determine if increased amounts of these molecules are expressed in either host brain or transplant vessels during graft revascularization, 2-3 week old rats had pieces of fetal neocortex (E18-21) or mature autonomic ganglia placed in either ventricle IV or directly into the parietal cortex. After postoperative survival times of 8 hours to 1 week, 6u paraffin sections were processed for PAP immunocytochemistry using antibodies to FN or LN. There was a marked increase in FN staining in brain vessels adjacent to parenchymal grafts at 24-48 hours, when graft revascularization occurs. Fetal neocortical transplant vasculature also stained more intensely for FN during the first week. Growing CNS parenchymal vessels, whether immature or regenerating, express more FN than uninjured, mature brain vessels, while LN appears unchanged. Supported by Am. Heart Assoc. and NS-17468.

457.8

EFFECTS OF TIME OF TRANSPLANT AND FGF ON CORTICAL TRANSPLANT SURVIVAL IN CAVITIES OF NEONATAL AND JUVENILE RATS. R.P. Sandor*, M.F. Gonzalez, J.E. Loken*, P. Walicke, C. Davidson*, I. Twoomey*, F.R. Sharp. (SPON: J.W. Sharp). UCSF Dept. Neurology, V.A. Med. Ctr., S.F., CA 94121.

Many factors influence neural graft survival. We undertook an experiment to study the effects of time of transplantation, host age and fibroblast growth factor (FGF) on transplant survival in rats. FGF was used in two pulses because of its reported stimulation of angiogenesis.

Long-Evans rats were divided into Group 1 newborns and Group 2 juveniles (100-150 gms). Following anesthesia cavities were made in the forelimb motor cortex bilaterally in all rats. One cavity per rat, chosen randomly, was filled with basic FGF (500 ng/0.3 ml tris buffered saline). Each group was then subdivided as to whether they received a transplant immediately (day 0), or after 3 or 7 days. Donor frontal cortex was obtained from 16-18 day fetuses (Long-Evans). Just prior to implantation the cavities were filled a second time with basic FGF (500 ng).

Juveniles transplanted at day 0 had an overall graft survival of 30 days of 7%; those transplanted at day 7 had a survival of 76%. Grafts in newborns had survival rates of 16% and 75% at day 0 and day 7, respectively. Staining of brain sections was greater than normal with Nissl and less than normal with cytochrome oxidase and succinate dehydrogenase. Basic FGF appeared to have no effect on graft survival as used in this experiment.

457.9

SCANNING ELECTRON MICROSCOPY OF THE SURFACE OF FETAL BRAIN IMPLANTS INTO THE LATERAL VENTRICLE FOLLOWING HIPPOCAMPAL LESIONS. D.J. Paul*, R.H. Baisden and M.L. Woodruff. East Tenn. St. Univ. Col. of Med. Johnson City, TN 37614.

Mesopallium from day E-16 fetuses was implanted into cavities produced by hippocampal aspiration in rats. The implants established interfaces with the host brain parenchyma at ventricular and lesion surfaces. SEM revealed that, rather than the normal variation in ventricular surface features (dense cilia and/or microvilli), the implant surface is a matrix of interwoven cells and processes. The cells appear to give rise to the matrix of neuritic processes. Smooth oval cell bodies can be found individually and in small groups interspersed across the matrix. These cells resemble cultured ganglion cells (neurons) or oligodendroglia. Other cells are flattened and have course surfaces and resemble cells in brain aggregate culture. There are sparsely distributed regions with typical appearing cilia. Clumps of cilia can also be observed arising from shafts of some neuritic processes. In addition, there are regions of microvilli similar to regions found on the normal ventricular surface. The transplants appear to have surface features similar to those described in CNS cavities formed in response to pathological conditions such as syringomyelia. (Supported by USPHS Grant #ES04070 to MLW).

457.10

MAGNETIC RESONANCE IMAGING OF FERRITE - LABELED NEUROTRANSPLANTS. **D. Smith[1]*, L. Clarke[2]*, G. Arendash[3] (Spon: D. Cahill). Dept. of Surgery (Neurosurgery)[1], Radiology[2], Biology[3], Univ. of So. Florida, Tampa, FL 33612.**

Our laboratory has devised a technique for labeling neurotransplants with a colloidal gold marker which is apparently innocuous and persistent within grafted cells (Science, 239: 635-637, 1988). The present study employs a superparamagnetic substance as a marker to enable magnetic resonance imaging of labeled transplants in situ.

Ten-14 days after excitotoxic NBM lesioning, Sprague-Dawley rats received a 3 microliter unilateral transplant of ferrite-labeled (D-17) NBM cell suspension into the host's lesion. Animals underwent MRI scanning 1-2 mos. post-transplant, and their brains were histologically examined. The transplant was revealed as a clear region of signal void in T_1 and T_2-weighted images and was corroborated histologically. Control animals with NBM lesions alone and labeled but non-viable transplants were also examined. The results suggest the feasibility of monitoring neurotransplants in vivo with this technique.

457.11

FETAL FRONTAL CORTEX TRANSPLANTS AND GM1 GANGLIOSIDE TREATMENTS FACILITATE RECOVERY OF BRIGHTNESS AND PATTERN DISCRIMINATION IN ADULT RATS WITH VISUAL CORTEX LESIONS. D.G. Stein, E. Curran*, L. Orphanides*, B. Levin & R. Labbe* Clark U., Worcester, MA 01610 & V.A. Hosp. E. Orange, NJ 07019.

Adult rats received grafts of E19 frontal tissue into damaged occipital cortex 7 days after injury (TP). One group with lesions and transplants (TP+GM1), received injections of GM1 gangliosides (30 mg/kg) for 15 days and one group with lesions served as surgical controls (LC). These groups were compared to sham-operated rats on brightness discrimination (BD), pattern discrimination (PD) and spatial navigation (SN) performance in a water maze to assess transplant and ganglioside induced post-traumatic, behavioral recovery. We examined BD and PD learning to increasingly stringent criteria (e.g., from 9/10 successively correct to 15/16 successively correct for 2 consecutive days), and all of the injured animals were impaired with respect to sham controls; however, rats with TP & GM-1 performed better than the LC or TP groups. In contrast, we were surprised to note that on the SN task, the TP animals performed significantly better than TP & GM1 and LC groups. Uptake of C-2DG was measured during photic stimulation in area 18, 3 layers of superior colliculus, LGN and transplant. Despite behavioral recovery, no significant differences were observed in host tissue of the different groups except that shams had significantly higher uptake than brain damaged counterparts. Analysis of lesion size revealed no significant differences among the three lesion groups and rank-order correlations showed no significant relation between lesion size and behavioral performance for any of the measures employed. We conclude that under appropriate circumstances, grafts of fetal frontal tissue can partially restore visual functions in rats with severe injury to the occipital cortex. This research is supported by NIMH grant 9R01NS25685.

457.12

HOMOTYPIC FETAL TRANSPLANTS IN THE NEURON-DEPLETED SOMATOSENSORY THALAMUS : DEVELOPMENT OF GRAFTED NEURONS AND HOST MONOAMINERGIC AFFERENTS. F. Nothias*, J. Dusart*, B. Onteniente*', M. Geffard*² and M. Peschanski (spon : ENA) INSERM U 161 75014 Paris France, ' VA CNRS 339, Talence 33405 France, ² Lab. Biochimie, Bordeaux 33407 France.

Fetal thalamic neurons (E 15) implanted into the excitotoxically lesioned somatosensory thalamus grow, differentiate, and receive projections from host afferents (Peschanski and Isacson, J. Comp. Neurol, 1988). The present study was designed to analyze the time-course of development and morphological features of growing grafted neurons and ingrowing host-graft monoaminergic afferents.

Neuronal size and density were studied in Nissl-stained sections, using an image analyzer. The cross-section average area occupied by the soma of grafted neurons enlarges progressively from 65 μm^2 at day 7 post-grafting (7 dpg) to 185 μm^2 at 20 dpg, then remains constant. Neuronal density decreases in parallel to reach the adult level also around 20-25 dpg. At the EM level, most neurons exhibit various mo.rphologically immature features until 20-25 dpg, at which time myelinogenesis starts. A small number of neurons demonstrating various signs of immaturity are still observed at later stages. Synaptogenesis is almost absent at 7 dpg and the density of synapses increases rapidly over the following 2 weeks. Adult levels, however, are not reached before 60-90 dpg. Morphologically immature synapses are numerous at 10 and 15 dpg. Norepinephrin and serotonin-immunoreactive afferents originating from the adult host are already present in the transplants at 8 dpg. At this time, they have regained an immature morphology. After 15 dpg, monoaminergic afferents resemble normal adult fibers.

Compared to ontogenetic data, these results demonstrate that the development of grafted neurons and host-graft afferents resemble normal ontogenesis both morphologically and temporally.

457.13

EFFECTS OF NERVE GROWTH FACTOR (NGF) AND DEXAMETHASONE ON CATECHOLAMINE CONTENT OF RHESUS ADRENAL MEDULLA IN CULTURE. *A.C. Gore*, P. Claude & E. Terasawa.* Neurosciences Training Prog. & Wis. Reg. Primate Res. Ctr., Univ of Wisconsin, Madison, WI, 53715-1299.

Adrenal medulla is commonly used as a source of catecholaminergic tissue for neural transplantation. We thought it might be advantageous to culture medullary tissue prior to transplantation in medium containing NGF or dexamethasone (dex), since NGF, which facilitates neurite outgrowth, may shift the proportion of the 3 major catecholamines (CAs): norepinephrine (NE), epinephrine (E) and dopamine (DA); and since dex enhances cell survival. Therefore, we examined whether culturing medullary tissue in medium containing NGF, dex, both NGF + dex, or neither, affected the proportion of CAs relative to fresh tissue. Medullary tissue was dissected from adrenal cortex and cut into approximately 1 mm^3 pieces. 4-5 pieces were frozen immediately (fresh). The rest were cultured in 2.5 ml of Medium 199 plus nutrients (control), or medium containing either NGF (100 ng/ml), dex (10^{-5} M) or both for 2-3 days or 12-14 days. CAs were measured by HPLC with electrochemical detection, and total protein was measured using the Bradford method. ***Results:*** 1) CA contents in fresh tissue, expressed as CA/total protein were: E, 4.55×10^{-2}; NE, 1.98×10^{-3}; and DA, 4.62×10^{-4}. 2) Following 2-3 or 12-14 days of culture, E and NE decreased by 50-60%, although NGF tended to prevent the loss of E and NE. 3) DA content was similar in fresh and cultured tissue. Therefore, it appears that culturing medullary tissue results in a decline in E and NE but not DA. It is possible that culturing may change the content of other substances which were not examined in this study, such as opioid peptides and acetylcholine. For the purposes of neural transplantation, the use of fresh tissue seems to maximize total CA content. Supported by NIH HD-11355, HD-15433 & RR-00167.

457.14

AN EXAMINATION OF THE ABILITY OF CORTICAL TRANSPLANTS TO REVERSE MEMORY IMPAIRMENTS PRODUCED BY LESIONS OF THE NUCLEUS BASALIS IN RATS. A.C. Santucci, V. Haroutunian, R. Gluck and K.L. Davis. Bronx VAMC & Mt. Sinai School of Medicine, New York, NY 10468.

Although cortical transplants have been reported to reduce memory impairments produced by nucleus basalis of Meynert (nbM) lesions, little information is available concerning those transplant parameters that optimize recovery. Accordingly, 7-10 days after bilateral nbM lesioning, rats received fetal cell-suspension transplants (ventral forebrain) to either 0 (nbM), 2 (2TR) or 4 (4TR) frontal cortical locations. Sham-lesioned animals (SH) without transplants were also included. A spatial memory task that required subjects to find a water-filled receptacle among an array of 6x5 receptacles was employed. Animals received one trial/day and trials & errors-to-criterion served as dependent measures. Relative to the SH group, nbM and 2TR subjects required more trials and committed more errors before attaining criterion ($ps<.05$). The 4TR and SH groups exhibited statistically equivalent trial and error scores ($ps>.10$), while the 4TR and nbM groups differed on the trial measure ($p<.05$). Finally, the 2TR group did not differ from nbM animals ($ps>.20$). Experiments in progress examine the generality of this transplant-induced alleviation of lesion-induced memory impairments. Histological and neurochemical analyses will be conducted at the completion of behavioral testing.

457.15

FETAL BRAIN TRANSPLANTS ACCELERATE SPONTANEOUS RECOVERY ON PASSIVE AVOIDANCE LEARNING IN AMYGDALA LESIONED RATS. M.A. Sánchez*, J. Pérez*, S. Alvarez* and F. Bermúdez-Rattoni (SPON:R. Tapia) Instituto de Fisiología Celular. UNAM.

It has been shown that the lesions of basolateral and central nuclei of amygdala disrupt retention of passive avoidance learning. Morover, it has been reported that amygdala lesioned animals showed spontaneous recovery in a previously lost learned task. In this work we demonstrated that fetal brain transplant can accelerate the spontaneous recovery in amygdala lesioned animals. Male Wistar rats were randomly divided in five groups. Four groups sustained large amygdaloid lesions and one remained as an unoperated control group. All animal were trained to avoid foot-shock in a shuttle box, follow by five retention trials. After training two groups of lesioned rats received homotopic fetal brain transplants in the amygdala (TA), the other two groups remained as lesioned control groups (LxA). The TA and LxA animals were assigned in two pairs of groups. One pair of groups were retrained one month and the other two months later. The results showed that all animals tested aiter two months recovered the ability to learn the task. However, those grafted animals retrained one month later singnificantly improved the retention of the learned task as compared with the lesioned group. These results suggest that there are spontaneous recovery in amygdala lesioned rats in a passive avoidance learning task, and this recovery can be hastened by means of the grafted tissue.

457.16

A GOLGI AND SILVER IMPREGNATION STUDY FOR FETAL BRAIN TRANSPLANTS IN THE GUSTATORY NEOCORTEX. M.L. Escobar*, S. Díaz-Cintra, A.L. Piña-Hernández*, L. Cintra and F. Bermúdez-Rattoni. Inst. de Fisiología Celular and Inst. de Invest. Biomédicas, UNAM, México, D.F. 04510.

Recently we have demonstrated that the fetal brain transplants produced recovery of taste aversion learning in adult rats with gustatory neocortex (GN) injury (Brain Res. 416:147-152,1987). In this study we showed by Golgi and argentic histological analysis, that fetal brain transplants produced anatomical recovery. Rats previously GN lesioned, received neural tissue transplantation from fetuses of 17 days old in the lesioned area. After eight weeks of recovery all animals underwent to a Golgi and silver impregnation techniques. The silver impregnation showed astrocytes, neuronal somas and fibers in the border of the transplant as well as fiber bundles into the transplant. We found a neural reorganization in both tissues, with a more neuronal density in the transplanted tissue. With the Golgi method some neurons were well impregnated in the transplant, and was possible to identify multipolar neurons in advanced state of differentiation. We can affirm that the fetal transplant could adhere to the host tissue with abundant vascularization, a great proliferation of glial cells in the transplant's border, as well as fibers that penetrates from receptor tissue to transplant. This indicate a dynamic process of morphologic and functional interrelation between the transplant and the host tissue.

457.17

FETAL BASAL FOREBRAIN GRAFTS TO BASAL FOREBRAIN AND NEOCORTEX: EFFECTS OF DONOR AGE AND TARGET. R.J. Mandel[1,2], L.J. Thal[1,2], & F.H. Gage[2]. 1. Dept. Neurol. VAMC, SD, 92161, 2. Dept. Neurosci., UCSD, La Jolla, 92093.

Grafting of fetal basal forebrain neurons into the brains of rats with previous lesions of the nucleus basalis magnocellularis (NBM) may reverse observed learning and memory deficits. We investigated the influence of target and age of donor tissue on parameters of graft survival.

F-344 rats (n=27) were lesioned bilaterally with ibotenate in the NBM. Three weeks post-surgery rats received either, cortical injections of E-11 fetal cell suspension (n=4), cortical injections of E-16 suspension (n=5), NBM injections of E-11 suspensions (n=4), or NBM injections of E-16 suspensions (n=5). Nine lesioned rats served as lesioned controls (LC) and 5 sham rats served as unlesioned controls (C). Five weeks post-grafting, the rats were tested in a water maze paradigm (1 trial block/day, 2 trials/block, 10 total trial blocks). All the grafted animals and 2 lesioned controls were perfused transcardially and their brains processed for AChE histochemistry and cresyl-violet staining. The remainder of the LC and C rat's brains were assayed for cortical choline acetyltransferase (ChAT) activity.

The LC rats had a moderate cortical ChAT depletion (-27%) and their lesions were histologically similar to the rats with cortical grafts. All lesioned animals were impaired relative to C rats in performance of the water maze task and none of the grafting treatments affected maze behavior relative to LC rats. All grafts placed in the NBM survived (both donor ages), while less than 50% of the cortical grafts survived in either donor age group. In the NBM, E-11 suspension graft volumes were 3 times larger than E-16 suspension grafts. There was no effect of donor age on cortical graft volume but all cortical graft volumes were significantly smaller than comparable grafts to the NBM.

Both age and target are important variables for graft survival of fetal basal forebrain.

457.18

HOMOTYPIC FETAL TRANSPLANTS IN THE NEURON-DEPLETED SOMATOSENSORY THALAMUS ; ULTRASTRUCTURAL EVIDENCE OF RECONSTRUCTION OF THE DEFECTIVE CIRCUITRY. M. Peschanski, F. Nothias*, O. Isacson*[1], B. Onteniente*[2], M. Geffard*[3] and F. Roudier* INSERM U 161, 75014 Paris France, [2] Dept of Anatomy, Univ. Cambridge England, [2] UA CNRS 339 33405 Talence France, [3] Lab. Biochimie cellulaire et de neurochimie, 33407 Bordeaux France.

The neural circuitry of the somatosensory relay nucleus of the rat thalamus is relatively simple in that all afferents can be grouped in only four morphological types and each of these types correspond either mostly or exclusively to fibers originating from one source. LR (large terminals, round vesicles, assymetrical synapses) = ascending somesthetic afferents; F (flat vesicles, symmetrical synapses, GABAergic) = thalamic reticular nucleus; SR (small terminals, round vesicles, assymetrical synapses) = mostly cortico-thalamic projections; nsV (vesicle-filled non synaptic varicosities) = monoaminergic systems.

After thalamic excitotoxic lesion, the various afferents remain in the area although they undergo morphological alterations (Peschanski and Besson, JCN 1987) and all synaptic arrangements, including vesicles, disappear. When homotypic fetal neurons (E 15) are transplanted and allowed to grow and differentiate within the excitotoxically lesioned area for several months, all types of terminals (LR, F, SR, nsV) are observed in the transplant. We have labeled the main afferent systems using either the anterograde transport of WGA-HRP (for ascending somesthetic and cortico-thalamic afferents) or immunocytochemistry (for GABA, 5 HT and norepinephrin). Results demonstrate not only that all these elements of the normal somatosensory thalamic circuitry are present in the transplant but also that they have regained their usual terminal and synaptic morphology. There is, thus, a reconstruction of the defective thalamic circuitry. Additional data demonstrate, however, that other ultrastructural features are at variance from normal.

457.19

COMPARATIVE ANALYSIS OF PEP-19 AND 28-kDa Ca-BINDING PROTEIN EXPRESSION IN PURKINJE CELLS GRAFTED TO pcd MUTANT CEREBELLUM. A.C. Chang*, C.J. Alvea*, L.C. Triarhou, W.C. Low and B. Ghetti. (SPON: A.N. Siakotos). Depts. of Pathology (Neuropathology) and Physiology & Biophysics, and Program in Medical Neurobiology, Indiana Univ. Sch. of Med., Indianapolis, IN 46223 and Inst. of Neurosciences, National Yang-Ming Med. Coll., Taipei, Taiwan, R.O.C.

Cerebellar suspensions prepared from normal mouse embryos (E12) were grafted to the cerebellum of 45 day-old 'Purkinje cell degeneration' (pcd) mutant mice which, by that age, have lost virtually all of their Purkinje cells (Pc). Graft development was monitored at various times by the use of antibodies against two proteins localized selectively to Pc in normal cerebellum: 28-kDa Ca-binding protein (CaBP) and 7.6-kDa PEP-19 (gifts of M.R. Celio and J.I. Morgan respectively). CaBP immunoreactive cells were detected in the host molecular layer 9 days after transplantation (corresponding age of grafted Pc neonatal) and persisted throughout the survival time allowed (3 months). PEP-19 immunoreactivity was evident as early as 5 days after transplantation (corresponding age of grafted Pc E17) and persisted throughout the survival time allowed as well. The morphological development of immunoreactive Pc appeared very similar with both antibodies. Following grafting, somatic thorns were present at 9 days and elaborate dendritic arbors from 17 days on. These results indicate the ability of grafted Pc to retain features of normal chemical differentiation.

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457.20

FETAL MEDIAL HABENULA (MH) TRANSPLANTS: INNERVATION OF INTERPEDUNCULAR NUCLEUS (IPN). N.J. Lenn & G. Raisman. Dept Neurol, Univ of Virginia, Charlottesville 22908 & Natl Inst for Medical Research, London, England.

Several protocols were tested in which important transplantation variables differed. Donor tissue was the medial-dorsal lip of III ventricle from fetuses of the same inbred rat strains as the hosts. Explants were cultured for 1-2 days, with colloidal gold conjugated to WGA (Seeley & Field, '88) to label the cells. 1-45 days later rats were anesthesized and perfused with saline for slices, formalin for LM, or mixed aldehyde for EM.

20% of 23 cases with E19 fetal explants contained donor neurons compared to 75% of 22 cases with E16 donors; the latter were markedly larger. MH neuron birthdays are E15-18, by E19 both nuclei are fully demarcated and some MH axons have reached IPN. 3 of 3 cases with HRP injected into host IPN had retrograde labeled neurons in the transplant. This occurred without deafferentation of the host MH afferents, and might be increased with deafferentation. Colloidal gold labeled macrophages, some oriented capillaries and GFAP+ processes marked the donor-host interface. Numerous synapses in the transplant were not unusual in any way.

This protocol will permit testing of hypotheses regarding the molecular bases by which the distinctive synaptology of IPN develops. Supported in part by grants NS 16882 & FO6 TW01294 (N.J.L.) & by MRC (G.R.).

MOTOR SYSTEMS II

458.1

QUANTIFICATION OF REACHING MOVEMENTS IN SUBJECTS WITH SPASTICITY. L.Fetters & J. Kluzik*. Department of Physical Therapy, Boston University, Boston, MA 02215.

Kinematic data were used to quantitatively describe components of reaching movements(e.g.,smoothness)traditionally described qualitatively. Five,7 to 12 year old children with spasticity and 5 aged matched controls reached to target. The WATSMART three dimensional motion analysis system and video were used for analysis.

Dependent variables included displacment of the hand and number of acceleration/decelerations during reach to target. A single accleration/deceleration combination was a movement unit (L.Fetters & J. Todd, J.Motor Behav.,19:2,1987).

Reaches of the children with spastic quadriplegia included significantly more movement units than children with normal motor abilities. In addition, the number of movement units decreased following exercise with the spastic subjects.

The analysis of movement units (stop/start action) is a viable method for characterizing reaching movements and for assessing change in subjects with motor disability.

458.2

DEPRIVED EARLY SOMATOSENSORI-MOTOR EXPERIENCE IN STUMPTAILED MONKEY NEOCORTEX: DENDRITIC SPINE DENSITY AND DENDRITIC BRANCHING OF LAYER IIIB PYRAMIDAL CELLS. Guy K. Bryan and Austin H. Riesen. Div. Biomedical Sciences and Psychology Dept., University of California, Riverside, CA 92521.

Macaca arctoides were individually raised to age 6 months in large clear cubes built into one wall of a control colony, allowing visual access to it but not tactile contact. Two deprivation conditions examined (Cond 2 and Cond 3) were equal both in physical size and with respect to partial social isolation. They differed in the amount of somatosensori-motor opportunity available during development in that the Cond 2 chamber was empty, whereas Cond 3 contained ladders, and a trapeze. Four monkeys from each of these conditions were compared with four colony-reared (Cond CR) monkeys. The neuroanatomical changes resulting from these rearing conditions were assessed by counting dendritic spines on the apical shafts of layer IIIB pyramidal cells in MI, SI, and V1 cortical regions as seen using light microscopy in Golgi-Cox stained tissue. Layer IIIB pyramidal cells with somas of medium size were selected for analysis; a sample of 10 such neurons was gathered from each cortical region and the density of apical dendritic spines determined. In addition, the basilar dendritic branches of these same neurons were traced, and dendritic branching complexity assessed in order to directly compare the sensitivity of the dendritic spine and branching measures. We found that apical dendritic spine density was significantly reduced in Cond 2 when compared with either Cond 3 or Cond CR (which did not differ from each other). This occurred in both MI and SI cortex, but did not in V1 cortex, the region used as a control for a generalized brain effect. Branching complexity on the same pyramidal neurons was reduced only in MI cortex of Cond 2. These results show spine density, a more direct measure of neuronal connectivity, to be the more sensitive measure of early environmental deprivation. Also, the enriched environment provided by Cond 3 relative to Cond 2 offset the effect of partial social isolation such that both morphometric measures were comparable to Cond CR monkeys.

458.3

POSTNATAL MATURATION OF THE PALLIDUM IN MONKEYS. J. Cano*, P. Pasik and T. Pasik. Depts. of Neurology & Anatomy, Mount Sinai Sch. Med., CUNY, N.Y.C., N.Y. 10029.

The pallidum of 20 rhesus monkeys, newborn to 4 months in age, was examined in Golgi material and ultrastructurally. All neuronal types seen in the adult are found at birth. The most common large fusiform cell shows initial signs of immaturity: blunt protrusions and dendritic dilations at bifurcation points, growth cones, filopodia and filiform processes. By 4 months, they appear fully mature save for underdeveloped terminal dendritic arborizations. The large globular cells and the interneurons are more mature than the previous type at all ages. The afferent radial fibers of striatal origin are observed from birth. They form bundles only after 8 weeks, and the density of their climbing branches increases over time reaching a mature appearance by 16 weeks. Afferents entering from the ventral surface do not show yet clusters of varicosities at 2 weeks. At this age, plexi of fine beaded fibers cover large extensions of the nucleus.

Ultrastructurally, the basic neuropil organization is present at birth albeit with immature features: incomplete covering of the dendrites with axonal boutons, low level of myelination of radial fibers, presence of growth cones and degeneration profiles. Initially, most dendrites show large varicosities and protrusions, which can be postsynaptic to multiple terminals. The other dendritic type, with only an occasional axodendritic synapse, is also seen from birth and increase in size with time. The type I axonal boutons, probably of striatal origin, are immature at birth with their interdigitations showing only after the first week. The types II-V boutons appear mature at all ages examined. Crest synapses formed by type III terminals, are observed in the later stages. Finally, postsynaptic vesicle-containing profiles are present at 4 weeks, but triadic synaptic arrangements are apparent only by 16 weeks.

Results indicate the occurrence of progressive neuronal and neuropil changes in the monkey pallidum during the first 4 postnatal months, and suggest a faster rate of maturation than that of the neostriatum, probably as a reflection of its diencephalic origin. Aided by NIH Grants #NS-22953, NS-18657 and NS 11631.

458.4

AMPHETAMINE-FACILITATED RECOVERY OF BEAM-WALKING: DIFFERENCES IN MOTOR RECOVERY ARE NOT EXPLAINED BY LESION SIZE OR DEPTH. L.B. Goldstein, K.A. Walton*, and J.N. Davis. V.A. & Duke Medical Centers, Durham, N.C. 27705.

Treatment with amphetamine increases the rate of recovery of beam-walking in rats after a unilateral suction-ablation lesion of the sensory-motor cortex. While investigating this effect, we noted that several rats which had received amphetamine unexpectedly did not recover while several controls which were not given amphetamine rapidly recovered normal beam-walking performance. The present studies were performed to determine whether the motor recovery of these outliers was related to differences in lesion anatomy. The brain lesions of four groups of selected rats were characterized: 1. Amphetamine/ recovered (n=6), 2. amphetamine/ not recovered (n=6), 3. saline/ recovered (n=6), and 4. saline/ not recovered (n=6). There were no significant differences in lesion volume index (ANOVA, p=0.8), medial cortex damage (Kruskal-Wallis H=1.9, p>.05), lesion depth overlying the striatum (Kruskal-Wallis H=5.5, p>.05), or lesion depth overlying the hippocampus (Kruskal-Wallis H=0.2, p>.05) between the groups. None of the animals had a lesion of the underlying striatum whereas two rats in groups 1, 2, and 3 and one rat in group 4 had minor lesions of the underlying regio superior of the hippocampal formation.

These data show that differences in the motor recoveries of these selected groups of rats did not correlate with lesion volume, depth, or hippocampal damage. The lack of correlation of recovery with lesion size is consistent with observations of recovery of function in man.

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458.5

ELECTRICALLY INDUCED LOCOMOTION IN THE DEAFFERENTED *IN VITRO* BRAINSTEM-SPINAL CORD PREPARATION. Y. Atsuta*, E. Garcia-Rill and R.D. Skinner, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Recently we described the ability to induce adult-like, coordinated stepping following electrical stimulation of the brainstem in the hindlimb-attached, *in vitro* brainstem-spinal cord preparation (Atsuta *et al*, Anat. Rec. 220, 1988). These findings suggest the presence at birth of supraspinal systems capable of controlling spinal locomotion pattern generators. The present study employed the hindlimb-attached *in vitro* brainstem-spinal cord preparation from 0-4 day old rats maintained in oxygenated artificial CSF. After establishing the control threshold/frequency relationship, the dorsal roots to the attached limbs were severed and the procedure repeated. No changes in threshold or qualitative differences in the locomotor pattern were observed after dorsal root section. The mean frequency of alternation induced before deafferentation was 0.37 ± 0.6 Hz and, after deafferentation, was 0.43 ± 0.12 Hz. In some cases, remarkably fast alternation was seen only after deafferentation. There was an average increase of 13% (not statistically significant) in the frequency induced at the same electrical threshold following deafferentation. These results suggest that, 1) the supraspinal control of spinal oscillators is not dependent on afferent input, and 2) afferent input, in some instances, may limit the maximal frequency of alternation of the limbs. Supported by USPHS grant NS 20246.

458.6

DENSITY OF ACETYLCHOLINE RECEPTORS IN CAT TRICEPS SURAE MUSCLES AFTER SPINAL CORD ISOLATION OR VENTRAL ROOT SECTION, L. Eldridge, L.L. Bambrick* and T. Gordon, Dept. Physiol.U.C.L.A., Los Angeles, CA 90024; Dept. Pharmacology, Univ. of Alta., Edmonton, Alta., Canada, T6G 2H7.

Comparison of numbers of ^{125}I-bungarotoxin binding sites per mg protein was made in homogenate preparations of cat triceps surae muscles, 3 weeks to 8 months after (1) spinal cord isolation and deafferentation or (2) ventral root section of the contributing motoneurones. Numbers of binding sites were significantly elevated in all muscles at 3 weeks after surgery. However they returned to normal by 8 months in the muscles with intact motor innervation (1) in contrast to the denervated muscles (2) in which the numbers remained elevated. EMG recordings showed that all muscles fibrillated within the first month after surgery but this activity ceased in the muscles with intact innervation by 2 months. These results provide evidence that acetylcholine receptors are down-regulated in inactive muscles with intact motor innervation suggesting that intact but silenced motoneurones can regulate extrajunctional receptor density in inactive muscles.

Supported by AHF and NIH Grant (5R01AG02562-03).

458.7

MUSCLE HETEROGENEITY IN MOTOR UNITS IN REINNERVATED CAT AND RAT HINDLIMB MUSCLES, V. Rafuse*, T. Gordon, T.P. Martin*, S. Erdebil* and J. Totosy de Zepetnek*. Depts. of Pharmacology and Physical Therapy, Univ. of Alberta, Edmonton, Alta., T6G 2G7.

Reinnervating motoneurones specify the force output of denervated muscle fibers after complete and partial nerve injuries to restore the normal size relationships, but whether each motoneurone fully changes properties of the muscle fibers it supplies is uncertain. In correlative electrophysiogical and quantitative histochemical studies of motor units in cat triceps surae and rat tibialis anterior muscles we have found that discrimination of fiber types according to contractile speed, "sag" and fatiguability becomes far less reliable after reinnervation. An increased proportion of units become intermediate in fatiguability and the normal property of fast units to sag during unfused tetani, is not a reliable criterion for fast units. Histochemically, muscle fibres are readily classified into types whose proportions are not changed by reinnervation but whose distribution in the muscle is clustered rather than widely distributed. Initial observations that the metabolic properties of muscle fibers within a motor unit are significantly more heterogeneous in character than they are normally may account for loss of sharper distinctions between motor unit types after reinnervation. Supported by the MDAC, MRC and AHFMR.

458.8

BILATERAL PROJECTIONS FROM MOTOR CORTEX TO THE NUCLEUS OF DARKSCHEWITSCH AFTER NEONATAL OR ADULT HEMISPHERECTOMY. R.L. Sutton and J.R. Villablanca. Depts. of Psychiatry and Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.

The descending cortical pathways to the n. of Darkschewitsch (ND) were examined in cats with neonatal (N) or adult (A) left cerebral hemispherectomy (HEMI) after injection of [^{3}H]-leucine-proline into the remaining motor cortex. Computerized analyses revealed that autoradiographic grain densities in the left ND of adult cats with N-HEMI reached values ranging from 63-100% of that measured in the right ND while these values ranged from only 11-28% for A-HEMIs (vs. 3-9% for intact controls). After injection of WGA-HRP into the intact motor cortex of adult cats with N-HEMI, electronmicroscopy revealed peroxidase-labeled terminals synapsing on ND neurons bilaterally. Fibers of origin to each ND appeared to ascend through the ipsilateral red n. (RN). These age-at-lesion effects on the decussating cortical projection to the ND parallel those published for the RN and support a proposed ND-RN relationship. Supported by Grants USPHS R01 NS 25780, HD-05958, and HD-07032.

458.9

SPARED DESCENDING PATHWAYS CONTRIBUTE TO THE RECOVERY OF BIPEDAL BUT NOT MONOPEDAL MOTOR FUNCTION AFTER HEMISECTION IN THE ADULT CAT. M.E. McBride and M.E. Goldberger. Medical College of Pennsylvania, Phila., PA. 19129.

Recovery of locomotion and reflex activity following hemisection occurs in precise stages, suggesting that different pathways mediate recovery of discrete monopedal responses and bipedal activity. Spared descending pathways or primary afferent input may be responsible. To test the contribution of descending systems to the recovered behavior, a contralateral 2nd hemisection was made 4-5 segments rostral to, and 6 months after the initial hemisection. If spared descending pathways were responsible for recovery after the 1st hemisection, a decompensation of motor function would be expected after the 2nd hemisection. Threshold and kinematic measurements for postural responses showed no decompensation of monopedal responses. In fact, a significant decrease in thresholds were measured for monopedal hopping and placing on the chronic side accompanied by a progressive recovery of postural reflexes on the acute side. Kinematic analysis of conditioned overground and treadmill locomotion did decompensate but recovered again; accurate placement during precise locomotion did not recover. These results suggest that contralateral descending systems are not responsible for recovery of monopedal responses but do contribute to complex bipedal activity necessary for accurate limb placement.

Supported by NIH grants NS24707, NS16629, & NSF grant NS8605441.

458.10

ENVIRONMENTAL ENRICHMENT DURING GROWTH AFFECTED BODY SIZE AND SHAPE OF GERBILS. MaryLou Cheal, Cheryl A. Alongi*, and Mary W. Marzke*. Depts. Psychology & Anthropology, Arizona State University, Tempe, AZ 85287.

Environmental enrichment facilitated rapid growth in adolescent gerbils (Cheal, 1984). In order to maximize potential differences during earlier growth, gerbils (n = 35) were born and group reared in large cages (35 X 45 X 66 cm) with locomotor incentives, or in small rat cages (18 X 20 X 25 cm). Body weight, body length, body segment lengths, and ratios of segment length/body length were recorded on alternate days from birth to 60 day and at 8 mo of age. From 2-4 wk, when locomotion increases dramatically and pups are weaned, enriched gerbils had longer body segment lengths than controls. Between 7-9 wk, body segment lengths and forearm length/body length ratios were greater in the enriched gerbils. By 8 mo, significant differences unrelated to allometric effects remained only in forearm length/body length. It was concluded that absolute size differences due to environmental enrichment may be short-term, but forearm/body length proportions may be permanently affected.

458.11

POSTNATAL DEVELOPMENT OF DOPAMINERGIC SYSTEMS IN RAT STRIATUM; W. C. Broaddus and J. P. Bennett, Jr.;Depts. Neurosurgery, Neurology and Neuroscience, University of Virginia, Charlottesville, VA 22908.

The prominent "matrix" component of dopaminergic input to rat striatum from substantia nigra is absent at birth, and appears progressively during the first 3 to 4 weeks of life. This developmental paradigm provides a model for studying striatal cells before, during and after acquisition of afferent dopaminergic input.

Neonatal rats were sacrificed and their striata homogenized and washed twice by centrifugation. Binding studies with [3H]-SCH23390 revealed a single class of D_1 receptors (K_d 0.45 ± 0.24 nM). No significant change in K_d was noted during normal postnatal development. B_{max} increased from 17 to 80 pmol/gram(wet weight) between weeks 1 and 3, and then stabilized. [3H]-sulpiride binding revealed D_2 sites with a K_d of 3.1 ± 1.4 nM. Like the D_1 sites, B_{max} increased approximately 4-fold, to a plateau value of 7 pmol/gram at 3 weeks. Commensurate increases in B_{max} maintained a D_1 /D_2 receptor ratio of 9 to 12 throughout the postnatal period.

Specific [3H]-GppNHp binding revealed a K_d of 160 ± 10 nM, and a B_{max} which increased postnatally. Unlike D_1 and D_2 receptors however, this value continued to increase after 3 weeks of age. In adult animals the value was 10380 pmol/gram, suggesting an excess of specific guanine nucleotide sites of 130-fold relative to D_1 sites, and 1500-fold relative to D_2 sites.

Dopamine- and forskolin-dependent adenylate cyclase activities increased approximately 4-fold between 1 and 3 weeks, and then reached plateau values of 5 and 115 pmol/min/mg (wet weight), respectively. Basal, guanine nucleotide-, and Mn^{2+}-stimulated adenylate cyclase also increased postnatally, but reached plateau values earlier. An increase of only 1.5 to 2-fold was seen between 1 and 2 weeks, after which the activities stabilized close to adult levels.

Between 2 and 3 weeks a reproducible change in cyclase response to GTP was also revealed. Initially a simple sigmoidal dose-response relationship is evident, with maximal GTP levels eliciting maximal cyclase activities. Beginning at 3 weeks however, a biphasic response is seen, with maximal cyclase activity at 1 uM GTP. Above 1 uM, a progressive decline in activity is seen. Others have shown in adult rats that this biphasic effect is abolished by pertussis toxin treatment. This suggests that in neonates a transition in relative or absolute quantities of G-proteins occurs between 2 and 3 weeks of age. We speculate that this may be related to the arrival of nigrostriatal fibers, which occurs at the same time.

458.12

EFFECTS OF 6-HYDROXYDOPAMINE ON POSTNATAL DEVELOPMENT OF DOPAMINERGIC SYSTEMS IN RAT STRIATUM; J.P.Bennett, Jr. and W.C.Broaddus; Depts. Neurosurgery, Neurology, and Neuroscience; University of Virginia, Charlottesville, VA 22908.

Two protocols for intracerebroventricular injection of 6-OHDA were used to study development of the striatal dopaminergic system in neonatal rats (see also WC Broaddus and JP Bennett, accompanying abstract). One group of rats received bilateral injections once on postnatal day 3. This destroys dopaminergic input to striatal "patch" areas, but allows subsequent ingrowth of fibers to "matrix" neurons (Gerfen et al.,J Neurosci. 7, 3935, 1987). A second group of animals received injections at weeks 1, 2, 3, and 4, and were sacrificed at 7 weeks.

In the first group, striatal dopamine (DA) levels fell to 55% of control 1 week after the injection, but returned to approximately 75% of control thereafter. [3H]-Mazindol binding decreased to 30% of control at 1 week, but then rose to 130% of control, suggesting sprouting of the remaining dopaminergic terminals in response to lesioning. A 40% increase in the (DOPAC+HVA)/DA ratio was seen at 1 week, suggesting a compensatory increase in DA turnover. In the second group, DA was decreased to 32%, [3H]-mazindol binding to 70%, and (DOPAC+HVA)/DA increased to 170% of control values. Unlike the one-time injection however, these changes were persistent 3 weeks after the last (fourth) 6-OHDA injection.

Adenylate cyclase activities were characterized in each group: no changes in basal, guanine-nucleotide-, DA-, Mn^{2+}-, or forskolin-stimulated adenylate cyclase activities were found.

Dopamine receptor binding studies revealed a 50 to 60% increase in the apparent number of D_2 sites in 6-OHDA-injected animals, without any change in K_d. This change persisted in the first group of animals, despite near-normalization of other parameters. No changes were seen in D_1 receptors after 6-OHDA-injection with regard to antagonist affinity or number of sites. Agonist affinity was apparently preferentially increased however, as a 2 to 3-fold decrease in IC_{50} for SKF38393 was noted after single or multiple 6-OHDA injections. A GppNHp-dependent decrease in agonist affinity was also seen in both 6-OHDA and control animals, suggesting that these two phenomena may be independent.

Parallel behavioral studies, tissue autoradiography, and immunoblot analysis of second messenger system components are currently underway or planned to further characterize pre- and post-synaptic events in the development of nigrostriatal input.

458.13

THE ONTOGENY OF NAAG-LIKE IMMUNOREACTIVITY IN THE RAT SPINAL CORD. J. H. Neale, K. E. Miller, M. F. Humphrey and P. M. Sweetnam. Dept. of Biology, Georgetown University, Wash. D.C. 20057 and Dept. of Neurol. Surg. University of Miami School of Medicine, Miami, FL 33136.

The neuronal distribution, vesicular localization and depolarization-mediated release of N-acetylaspartylglutamate (NAAG) suggest a role for this dipeptide in transcellular communication. While the concentration of NAAG in the rat nervous system increases postnatally, the amount present in the newborn suggests the possibility of a trophic role for NAAG in prenatal development. To explore the function of this dipeptide, the development of NAAG-like immunoreactivity (LIR) was examined by immunohistochemistry in embryonic, early postnatal and adult spinal cord and dorsal root ganglia. As described previously, NAAG-LIR was present in adult spinal cord and spinal sensory neurons. Additionally, NAAG-LIR was detectable in mid and late embryonic as well as early postnatal neurons in these tissues. This relatively early appearance suggests that NAAG may have a role in development of the spinal-sensory axis. Support: DA 02297; The Miami Project to Cure Paralysis; Daniel Heuman Spinal Cord Research Foundation.

458.14

NUMBER AND SIZE OF AXONS INNERVATING THE MYSTACIAL MUSCULATURE OF THE RAT VIA THE BUCCAL BRANCH OF THE FACIAL MOTOR NERVE. P.W. Hinds*, C. Welt and J.H. Abbs (SPON: A. Weber). Speech Motor Control Labs., Waisman Center., University of Wisconsin, Madison, WI 53705-2280.

The highly ordered and behaviorally significant mystacial vibrissae system of rats provides a unique model to examine structural and functional reorganization following motor nerve damage. As a first step, electron microscopic and computer-assisted quantitative methods were used to determine the organization and fiber spectrum of axons innervating the mystacial muscles via the buccal branch (BN) of the facial nerve. In contrast to previous reports of only one branch in the BN, we consistently observed one large and two smaller branches. In five rats, the main branch had an average cross-sectional area of 52,240 µm2 and contained an average of 1543 myelinated (86%) and 260 unmyelinated (14%) axons. Fiber size (mean diameter including the myelin sheath) ranged from 1.97 to 9.67 µm, with population means of 4.61 to 6.37 µm (weighted ave.=5.35, s.d.=1.36). Axons were not arranged in separate fascicles or segregated according to size. The smaller branches showed a different quantitative profile. More than 75% of these axons were unmyelinated. The mean diameter of the myelinated fibers was smaller than in the main branch, ranging from 1.31 to 8.78 µm, with population means of 3.38 to 3.92 µm (weighted ave.=3.67, s.d.=1.63). In contrast to the normally distributed fiber sizes in the main branch, the size distributions were skewed to the smaller diameter fibers in the small branches. After applying HRP to the main branch, labeled cell bodies were found in the ipsilateral facial nucleus. In contrast, cell bodies for the small branches were in the trigeminal ganglion. These data were used to assess regeneration and recovery of function after facial nerve section. (Supported by NIH grants NS-13274 and HD-03352).

458.15

REINNERVATION OF MYSTACIAL VIBRISSAE AFTER FACIAL NERVE SECTION AND REPAIR IN RATS. C. Welt., P.W. Hinds*, M.H. Schutta* and J.H. Abbs. Speech Motor Control Labs., Waisman Center, University of Wisconsin, Madison, WI 53705-2280.

Reorganiztion of mystacial vibrissae innervation was studied in adult rats after facial n. (VII) section. Four methods, used to repair human facial n. damage, were analyzed. After cutting VII, the distal segment was (1)reattached to its proximal end, (VII-VII); (2)sutured to the proximal end of the cut hypoglossal n. (VII-XII); (3)sutured to the proximal end of the cut spinal accessory n. (VII-XI); or (4)allowed to regenerate without reattachment. After six months, HRP injections, quantitative electron microscopy of the buccal branch (BN) of VII, and observations of vibrissae movements were used to assess reorganization. Axons in the main branch of the BN regenerated and reinnervated the pad in all four groups. Two small branches, originating in the trigeminal ganglion, were unaffected by the VII n. section. HRP injections in the pad on the operated side labeled many cells ipsilaterally in the facial nucleus (FN) of VII-VII; the hypoglossal nucleus of VII-XII; the spinal cord of VII-XI, and the FN of unsutured VII. Labeled cells were not topographically arranged; neurons from all regions of each nucleus regenerated to the vibrissae. Quantitative profiles of the regenerated nerves were remarkably similar regardless of which nucleus was the source. Cross-sectional areas of the main branch were slightly smaller than normal, but they contained 2.70 to 3.25 times as many axons. Some fibers attained normal size, but mean fiber size was only 45-56% of normal, due primarily to smaller axon cylinders rather than to relative myelin thickness. Some vibrissae activity was present in all animals, but coordinated whisking did not return. At six months, however, behavioral changes and neural regenerative processes, such as remyelination, are still taking place. (Supported by NIH grants NS-13274 and HD-03352)

458.16

NIGRAL DA CELL RECRUITMENT AS A COMPENSATORY MECHANISM. J.R.Hollerman and A.A.Grace,Univ of Pittsburgh, Department of Behavioral Neuroscience, Pittsburgh, PA, 15260. Previous studies have shown that a significant proportion of nigral dopamine (DA) neurons are not spontaneously firing but can be activated by administration of a DA receptor blocker (Grace and Bunney, 1984). The present study investigates whether this population may also be recruited to compensate for 6-hydroxydopamine (6-HDA) induced striatal DA depletions.

DA depletions were produced by administration of 6-HDA bilaterally into the lateral ventricles following pargyline and DMI pretreatment. DA neurons were identified by previously established criteria (Grace and Bunney, 1983). Estimates of the relative number of active cells was made by counting spontaneously firing DA neurons using a 12 track protocol as previously described (Bunney and Grace, 1978). In DA depleted animals, this value did not differ from control unless striatal DA depletion exceeded 50%. The number of active cells after striatal DA depletions between 50 and 90% of control was also greater than the number predicted based on a linear decrease in spontaneously active cells relative to the extent of DA depletion. Although this provides evidence for the recruitment of inactive cells to compensate for the DA depletion, the actual proportion of cells activated is currently being investigated using histofluorescence techniques to establish the extent of nigral cell death relative to extent of striatal DA depletion. Supported by NS19608.

458.17

NEURONAL REORGANIZATION COINCIDE THE DEVELOPMENT OF CHEWING BEHAVIOR FROM SUCKING IN GUINEA PIGS. A. Iriki* and Y. Nakamura* (SPON: H. Asanuma). The Rockefeller University, New York, NY 10021 and Dept. Physiol., Fac. Dent., Tokyo Med. Dent. Univ., Tokyo 113, Japan.

It has been generally thought that conversion from sucking to chewing depends on a peripheral events, i.e., eruption of the teeth. However, this cannot be the case in guinea pigs which are born with mature oro-facial structures including complete permanent dentition. The conversion from sucking to chewing should therefore result from change in central mechanisms. We investigated this possibility in ketamine-anesthetized guinea pigs. To produce rhythmical jaw movements, continuous intracortical microstimulation was used. The following results were obtained: 1) Sucking was only induced in neonates, chewing only in adults. These were driven by different groups of cortico-bulbar neurons through rhythm generator in the brain stem. 2) Cortico-bulbar neurons which elicit sucking were located among the agranular cortex, while those which elicit chewing were located in the disgranular area of the granular cortex. The latter was newly formed during postnatal development. 3) During development, neurons of the agranular cortex lost their projection to the brain stem and became to activate the newly formed chewing area in the disgranular area. It is concluded that reorganizations in cortico-bulbar projections form the bases of the change in jaw movements.

458.18

EARLY METABOLIC DIFFERENTIATION OF THE RAT DIAPHRAGM. B.W.C. Rosser, R.M. Choksi*, A.M. Kelly* and P.M. Nemeth. Departments of Neurology and of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110; and Department of Pathology, University of Pennsylvania, Philadelphia, PA 19104.

The developing rat diaphragm was studied with quantitative fluorometric assays for key energy-related enzymes, with pyrophosphate gel electrophoresis for native myosins and with northern blots for myosin heavy chain (MHC) mRNA. Perinatal transitions were compared to those in rat extensor digitorum longus (EDL), gastrocnemius and soleus muscles. Whole muscle homogenates assayed for malate dehydrogenase, succinate dehydrogenase, B-hydroxyacyl-CoA dehydrogenase, 1-phosphofructokinase, lactate dehydrogenase, creatine kinase and adenylate kinase had higher activities in the diaphragm than in hindlimb muscles, from 3 days before to 7 days after birth. Individual muscle fibers in the 7 day diaphragm had enzyme activities close to adult levels for type I and IIa fibers, in contrast to the comparable maturation of the hindlimb muscles at 14 to 21 days. Consistent with this earlier development of enzymatic phenotype, mRNA for neonatal MHC was detected at 3 days prior to birth in the diaphragm as compared to 1 day prior in the gastrocnemius, and adult fast myosin was observed at 5 days after birth in the diaphragm as compared to 15 days in the EDL. The results demonstrate that contractile proteins and energy-generating enzymes of the rat diaphragm differentiate in advance of the hindlimb. This earlier timetable of differentiation appears to anticipate the vital function of the diaphragm.

Supported by NIH Grant RO1-DK38375 and HL15835.

458.19

DIAPHRAGM FIBER SIZE AND OXIDATIVE CAPACITY FOLLOWING PHRENIC DENERVATION OR INACTIVATION. W.Z. Zhan* and G.C. Sieck (Spon: E. Eldred). Dept. of Biomed. Eng., USC, LA, CA 90089.

The purpose of this study was to determine the changes in size and SDH activity of diaphragm fibers after denervation or blockade of phrenic nerve activity. 3 groups of rabbits were studied: 1) Controls; 2) Denervated (DNV); or 3) TTX-treated, phrenic nerve activity blocked by TTX. After 30 days, muscle sections were cut and analyzed histochemically. Fiber types were classified based on ATPase activity. Fiber SDH activity was quantified using a microdensitometric procedure implemented on an image processing system (IPS). Fiber size was also determined using the IPS. SDH activity of type I fibers was reduced in both DNV and TTX animals. SDH activity of type II fibers also decreased in DNV animals, but increased in the TTX group. In both DNV and TTX groups, the size of type I fibers increased. The size of type II fibers also increased in TTX animals, but decreased following denervation. We conclude that differences in the adaptive responses of muscle fibers following denervation vs inactivation are found primarily in type II fibers and may be related to the persistence of neurotrophic influences in the TTX group.

458.20

TRANSCELLULAR LABELING BY ORTHOGRADELY TRANSPORTED TRITIATED INTRAAXONAL MARKERS. T.Pittman* and D.Tolbert. (Spon: H. Cantor). Depts Anat., Neurobiol. and Neurosurgery, St. Louis Univ. Sch. of Med., St. Louis, MO 63104

Previous reports have documented the uptake of injected tritiated proline by glia and its subsequent translocation along axonal pathways. We report evidence of transcellular labeling of glia with secondary transport of labeled amino acids during the development of corticopontine projections in cats. A mixture of tritiated proline and leucine was injected into the primary somatosensory area of the cerebral cortex. The animals were sacrificed and their brains fixed and processed for autoradiography. Cortical axons in the ipsilateral pontine nuclei (PN) were heavily labeled. Silver grains were present over the neuropil and outlined the somatic profiles of PN neurons. Small dense clumps of silver grains were concentrated over cell bodies whose size suggested that they were glia. From the ipsilateral PN diffuse linear arrays of label could be followed through the contralateral middle cerebellar peduncle (MCP) and into the cerebellar white matter where they ended. This pattern of silver grains was strikingly different from that overlying labeled axons. Individually labeled axons were not seen in the MCP. These findings suggest the release of transported label by corticopontine synapses and the subsequent uptake of the label by glia with secondary transport, either transaxonally or through chains of glia, of labeled amino acids. This transcellular labeling was seen only in neonatal cats. Supported: NIH Grant NS20227.

EPILEPSY: SUBSTANTIA NIGRA AND AMYGDALA

459.1

MODULATION OF SEIZURE SUSCEPTIBILITY BY NEURONAL TRANSPLANTS. B.S. Meldrum, S. Patel and A.Fine# (SPON: D.Rasmusson). Dept. Neurology, Institute of Psychiatry, London SE5 8AF, U.K. and #Dept. Physiology and Biophysics, Dalhousie Univ. Med. School, Halifax, NS, Canada B3H 4H7.

Partial epilepsy, the most common form of seizure disorder, is often unresponsive to drug treatment, leaving surgical removal of the focus as the main alternative. Seizures induced in rats by i.p. administration of the muscarinic agonist pilocarpine provide a useful model of complex partial epilepsy. Susceptiblity to such seizures has been shown to be increased by destruction of the striatonigral GABA projection, and decreased by infusion of GABAergic agonists into the substantia nigra (SN), suggesting that this GABA-mediated outflow from the basal ganglia influences spread of the seizures. To test the possibility that transplantation of fetal neurons from the GABA-rich neostriatum, transplanted to the SN, could suppress pilocarpine-induced seizures, 80g male Wistar rats were given bilateral ibotenic acid lesions of the caudate-putamen, while similar animals had sham lesions. One week later, lesioned rats received either bilateral fetal striatal eminence (SE) grafts or control grafts of peripheral nerve (PN) to the SN pars reticulata;additional animals served as lesioned-only and sham-lesioned controls. Four weeks later, all received 1 mg/kg scopolamine followed by 300 mg/kg pilocarpine, and resulting seizures over 2h were scored 0-7. Lesioned-only animals had more severe seizures than sham-lesioned controls, whereas SE- as well as PN-grafted animals were not significantly different from sham-lesioned controls.

459.2

CHRONIC CAFFEINE MODULATION OF POSTICTAL PHENOMENA IN AMYGDALA KINDLED RATS. C. LUPICA*and R. BERMAN (Spon: M.M. KILBEY). Dept. Psychology and Neurosci. Prgm., Wayne State University, Detroit, MI 48202.

We have previously shown that ip and intrafocal injections of adenosine (ADO) receptor agonists can reduce the severity of kindled seizures. IP administration of these compounds can also result in an increase in the duration of postictal EEG and behavioral depression, and a decrease in postictal spiking frequency. The ADO antagonist caffeine (CAF) can block these above effects and, when given chronically, increases the number of ADO A1 binding sites. In the present experiment we reasoned that prolonged exposure to CAF would increase ADO receptor density and mimic ADO agonist effects on kindled seizures. Sixteen Long Evans rats were implanted with bipolar stimulating/recording electrodes in right amygdala. Once daily electrical stimulation was then delivered until animals were fully kindled. Eight rats were then injected ip with 35mg/kg/day CAF for 1 wk, followed by 55mg/kg/day for 2 wks. The remaining rats served as saline injected controls. One day following the final CAF injection rats were retested for seizure severity and postictal measures. Animals treated with CAF displayed less postictal spiking ($p<.05$) and extended postictal depression ($p<.05$) as compared to controls. No differences in seizure severity between groups were observed. These data support the involvement of the brain adenosine system in the postictal state. (Supported by NIH Grant RR-08167, and a W.S.U. Neuroscience Prgm Fellowship to C.L.).

459.3

THE INTERACTIVE EFFECT OF COCAINE AND HYPERTHERMIA UPON KINDLING. G.T. Livezey and S.B. Sparber, Dept. Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

Daily cocaine injections (Post, R.M., In *Cocaine and other Stimulants*, E.H. Ellinwood, Jr. and M.M. Kilbey, eds., New York: Plenum Press, pp. 353-372, 1976) and exposure to acute hyperthermia once every 3-6 days (Klauenberg, B.J. & Sparber, S.B., Epilepsia, 25(3):292-301,1984) have produced kindling in rats. As cocaine use can be combined in some individuals with acute hyperthermia brought on by vigorous excercise, fever, or passive recreation (hottubs, sauna), and since a commonality of mechanisms has been proposed to explain kindling by a variety of stimulus classes (Post, 1976), we began a comparative study of cocaine, hyperthermia, and their combination in the production of kindling. PHASE I: Four male and four female siblings each from eight litters, were divided into groups receiving 1) hyperthermia (45°C water bath for four minutes) 2) cocaine (30mg/kg) plus hyperthermia 3) "normothermia" (37°C water bath for four minutes, procedure control) and 4) cocaine plus "normothermia". Same sex, weight matched, sibling pairs from drug and nondrug groups were run concurrently in the bath. All treatments were administered daily from 45 days of age for 16 trials. 13/18 group 2 animals convulsed early (trials 1-10) and of these 8 died. No other animals displayed convulsions through trial 16. PHASE II: Three litters of males with surviving group 2 animals that convulsed were implanted with telemetric transmitters (Data Sciences, Rosedale, MN) for body temperature and EEG data collection by computer, and their behavior plus oscilloscope monitor of EEG was taped by VCR. These animals subsequently each received one trial of treatments 1,4, and 2 in that order. Group 2 animals displayed more severe convulsions, and one animal each from group 1 and 4 convulsed upon the first trial (treatment 1) indicating kindling had occured from prior experience.Data suggest that the interaction of cocaine with hyperthermia is an enhancement of the kindling rate and severity. Supported in part by USPHS Grant T32DA07097.

459.4

Infusions of an Excitatory Amino Acid Antagonist into the Substantia Nigra Evoke an Age-dependent Effect on Seizures. J.N.D. Wurpel, E.F. Sperber & S.L. Moshé, Dept. of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461

Previous studies indicate that infusions of muscimol (GABA-A agonist) in the substantia nigra (SN) are anticonvulsant in adult rats. Muscimol infusions in the SN of 16 day old rat pups produce a proconvulsant effect on flurothyl (FE) seizures. We have found differing effects with infusion of 2-amino-7-phosphono-heptanoic acid (AP7- an NMDA antagonist) into the SN of adult and 16 day old rats on FE-induced seizures.

Bilateral infusions of AP7 (22.5 pg) into the SN of adult rats increased seizure latency compared to saline infused controls [AP7 707 ± 16 sec (x ± S.E.], N=12. Sal 478 ± 17 sec, N=8; p<.0001). Infusion of AP7 dorsal to the SN had no significant effect on seizure latency [528 ± 17 sec, N=4].

Bilateral infusion of the same concentration of AP7 into the SN of 16 day old rat pups did not alter seizure latency compared with saline infused controls [AP7 497 ± 12 sec, N=11. Sal 509 ± 18 sec, N=7; p>0.05].

We hypothesize that the immature SN may exhibit altered pharmacologic response to compounds which are anti-convulsant in adult rats; related to differences in local receptor populations or nigral efferent pathways.

459.5

WIDESPREAD REGIONAL DEFICITS IN BRAIN NOREPINEPHRINE (NE) UPTAKE AND DOPAMINE β-HYDROXYLASE (DBH) ACTIVITY IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR). R.A. Browning, D.R. Wade*, G. Long*, M. Marcinczyk* and P.C. Jobe. Southern Ill. Univ. Sch. Med., Carbondale, IL and Univ. Ill. College Med., Peoria, IL.

GEPRs are known to have widespread regional deficits in brain NE and serotonin. We have shown previously that NE uptake by synaptosomes from inferior colliculus and cerebral cortex of severe seizure GEPRs (GEPR-9s) is decreased (Browning et al. Fed. Proc. 45:675, 1986). Because the Vmax for uptake in cerebral cortex was reduced while affinity was unchanged, we hypothesized that GEPR-9s have fewer noradrenergic nerve terminals than normal rats. To test this hypothesis further, we compared GEPR-9 and seizure resistant controls in terms of DBH activity (a marker for NE and epinephrine containing neurons) and synaptosomal NE uptake in various brain regions. Expressed as a percentage of the values for control rats GEPR-9 DBH activity and synaptosomal NE uptake, respectively were: cortex, 59%, 61%; hippocampus 63%, 69%; amygdala 64%, 52%; hypothalamus 75%, 71% and inferior colliculus 72%, 66%. Neither DBH activity nor synaptosomal NE uptake was altered in the cerebellum of GEPR-9 rats. The parallel reductions in DBH activity and NE uptake in GEPR-9s are consistent with our hypothesis that GEPR-9s have fewer noradrenergic nerve terminals than control rats in several brain regions. Supported by Deafness Research Foundation.

459.6

INTRANIGRAL MUSCIMOL IN ANIMAL MODELS OF PETIT-MAL SEIZURES. A. Depaulis*, O.C. Snead, M. Vergnes*, & C. Marescaux* (Spon: B. Will). Centre de Neurochimie du INSERM et CNRS U44, 67084, Strasbourg, France, and Department of Pediatrics, University of Alabama at Birmingham School of Medicine, Birmingham, Alabama 35233.

The substantia nigra (SN) has been implicated as an important structure in the propagation of generalized convulsive seizures; therefore the effect of intranigral muscimol was assessed in several models of generalized absence seizures in the rat including the pentylenetetrazole (PTZ), THIP, γ-hydroxybutyrate (GHB), and genetic model of spontaneous spike wave discharges (SWD). Preliminary results suggest that intranigral muscimol (2ng/side) significantly suppressed seizures in the PTZ, GHB, and SWD models. Further, no paroxysmal EEG discharges were observed in the SN as a result of intranigral muscimol injections.

These data suggest that low doses of muscimol in the SN suppress seizure activity in experimental models of petit mal in a manner similar to that seen in generalized convulsive seizures. However, it remains to be proven that GABAergic mechanisms in the SN are a common denominator for the generalized epilepsies in view of the proconvulsant properties of systemic GABAergic agonists in experimental absence seizures (Vergnes et al: Neurosci Lett 44:91, 1984).

459.7

AUTORADIOGRAPHIC LOCALIZATION OF REDUCED ALPHA 2 ADRENERGIC RECEPTOR BINDING IN KINDLED RATS. L.S. Chen and J.O. McNamara. Epilepsy Research Lab, Duke and VA Medical Center, Durham, NC 27705

Norepinephrine (NE) powerfully inhibits the development of kindling, an effect likely mediated through alpha 2 receptors on targets of NE neurons. McIntyre and Wong (1986) demonstrated reduced alpha 2 mediated inhibition of epileptiform bursting in amygdala-pyriform cortex slices of kindled rats. We therefore hypothesized that kindling produces a reduction of alpha 2 receptor and/or receptor coupled response. Quantitative radiohistochemical analysis of alpha 2 receptor were performed to test this idea. Male Sprague-Dawley rats were kindled by amygdaloid stimulation and sacrificed 24 hours after 3 class 5 seizures. Ten micron brain sections of kindled and control rats were thaw- mounted onto microscope slides, incubated with 2.5 nM [^{3}H]-para(3,5)-aminoclonidine ([^{3}H]- PAC) at 25°C for 45 minutes in the absence and presence of 10 μM phentolamin, rinsed, dried, apposed to Ultrafilm, exposed for 6 weeks, and viewed by computer-assisted microdensitometry. Measurements of specific [^{3}H]-PAC binding disclosed a significant reduction in the central nucleus of amygdala (26%, $p<0.05$) and pyriform cortex (24%, $p<0.05$), but no change in nucleus accumbense, pre-pyriform cortex or adjacent neocortex. The present data extend previous findings from this and other laboratories (Stanford and Jeffery 1985; Chen et al. 1987) by demonstrating a spatially specific reduction of agonist binding to alpha 2 receptors in kindled rats. This reduction may be a key molecular event underlying the attenuation of NE's inhibitory effects and might thereby contribute to the development of kindling.

459.8

NOREPINEPHRINE AND SUPPRESSED KINDLING WITH MASSED TRIALS. D. C. McIntyre, M. E. Kelly*, C. Dufresne* and N. Edson* (SPON: T. Picton). Department of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Norepinephrine (NE) is involved in the suppression of seizure development. This effect is seen dramatically in the kindling model as rapid seizure genesis after 6-OHDA degradation of the NE system. The kindling of convulsions is optimally achieved by stimulating once per day, while massed stimulation results in retarded seizure genesis. In the present experiment, we determined the effect of the alpha-2 NE antagonist, yohimbine, and the beta antagonist, propranolol, on the suppressed seizures during massed trial kindling. This was achieved by exposing the rat amygdala to a 60 Hz sine wave stimulus once every 5 min. at an intensity immediately above the local afterdischarge threshold. This procedure was continued for 50 stimulations. The experimental (1 or 10 mg/kg yohimbine; 1 or 10 mg/kg propranolol) and vehicle groups received their i.p. injection immediately following the 15th kindling stimulation, during clear seizure suppression. The rate of kindling over the next 35 trials was then determined. Both doses of yohimbine facilitated the appearance of generalized seizures, while propranolol completely suppressed evidence of kindling over this period. These data indicate that the alpha-2 NE receptors participate in the suppression of amygdala kindling during massed stimulation, while beta receptors provide a proconvulsive profile.

459.9

EVALUATION OF ROLE OF EPINEPHRINE IN SEIZURE REGULATION IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR). P.K. Mishra, J.W. Dailey, C.E. Reigel and P.C. Jobe, **Department of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, IL 61656.**

Sound-induced seizure susceptibility of the genetically epilepsy-prone rat (GEPR) has been partially attributed to an innate defect in indices of central noradrenergic and serotonergic function. This study was conducted to determine whether epinephrine, a possible central nervous system neurotransmitter, has any role in seizure regulation in these animals. The assay of epinephrine concentration in hypothalamus of developing and adult GEPRs as well as in peripheral organs of adult GEPRs was accomplished using high performance liquid chromatography. A significant elevation in its levels in the hypothalamus, heart and kidney of adult GEPRs was not found to be associated with the seizure intensity. Moreover, the difference in hypothalamic epinephrine content of the developing GEPRs did not correlate with their seizure intensity. LY87130, an inhibitor of the epinephrine synthesizing enzyme PNMT, reduced seizure intensity, perhaps by increasing extracellular norepinephrine. LY51614, a monoamine oxidase inhibitor which elevates intracellular epinephrine, did not alter seizure indices. Results of the present study thus indicate that epinephrine may not play a major role in the seizure regulation in GEPRs.

459.10

GEPR-9s DISPLAY A REDUCTION IN THE DISTRIBUTION AND STAINING INTENSITY OF DBH-I PROCESSES. J.C. Lauterborn and C.E. Ribak, Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

An excellent model of genetic epilepsy is the genetically epilepsy-prone rat (GEPR-9) which exhibits motor seizures in response to auditory stimuli. Biochemical studies have indicated that norepinephrine is present in lower levels in GEPR-9 as compared to non-seizing Sprague-Dawley (SD) rats. In this study, the immunocytochemical localization of dopamine β-hydroxylase (DBH), the synthesizing enzyme for norepinephrine, was compared between GEPR-9s and SD rats. Sagittal brain sections from paired GEPR-9s and SD rats were processed simultaneously using an avidin-biotin immunocytochemical technique. Brain regions caudal to the inferior colliculus, such as the cerebellum and locus ceruleus showed no differences in the distribution of dopamine β-hydroxylase-like immunoreactivity (DBH-I). However, differences in the distribution of DBH-I fibers were observed in more rostral brain regions including the central nucleus of the inferior colliculus, thalamus, hippocampus, piriform cortex, and somatosensory cortex. In these areas, the density, and often the intensity, of DBH-I processes were lower in GEPR-9s as compared to SD rats. In other cortical regions no differences were observed. These results provide anatomical data that are similar to previously described biochemical results of the norepinephrine system in the brains of GEPR-9s. (Supported by NIH Grant NS-15669).

459.11

A MEDIAN THALAMIC SYSTEM REGULATING SEIZURES AND AROUSAL J.W. Miller, C.M. Hall*, K. Holland* and J.A. Ferrendelli Departs. of Neurology and Pharmacology, Washington Univ. School of Med. St. Louis, MO 63110

The effects of small intrathalamic injections of the GABA agonist muscimol on pentylenetetrazol (PTZ) seizure thresholds and spontaneous behavior were determined in rats and compared with the effects of injections of the GABA transaminase inhibitor, gamma-vinyl GABA (GVG). Muscimol injections in the dorsal midline thalamus in the vicinity of the paraventricular, paratenial, interanteromedial and intermediodorsal nuclei, as well as the central medial nucleus facilitate PTZ myoclonic and clonic seizures, and also produce a sleep-like state. The more posterior of these midline injections also inhibited tonic seizures. Injections lateral, dorsal or ventral to this midline region had much less effect on both seizures and behavior. In contrast, GVG injections in the anterior medial thalamus elevate the threshold for all PTZ seizure types but have little behavioral effect. These results demonstrate that inhibition of descrete thalamic regions with muscimol can modify seizure expression but inhibition of larger thalamic regions, as with the GVG injections, is required for a general anticonvulsant effect. Supported by NIH Grants NS14834, NS07129, GM07200 and the Seay Neuropharmacology Research Fellowship.

459.12

DORSAL HIPPOCAMPAL STIMULATION SUPPRESSES AMYGDALOID KINDLED SEIZURES. Linda J. Burdette, Ph.D. and Marc A. Dichter, M.D., Ph.D., Department of Neurology, Graduate Hospital and the University of Pennsylvania, Philadelphia, PA

Temporal lobe epilepsy, the most prevalent form of adult seizure disorder, is also the most resistant to treatment. The efficacy of electrical stimulation as a therapeutic alternative to ablative surgery in suppressing temporal lobe seizures was investigated in this study.

Male Long Evans rats were implanted ipsilaterally with bipolar electrodes in the basolateral amygdala and the dorsal hippocampus. Rats were kindled twice daily (200 μA, 2 second train of 50 Hz, .3 ms bipolar pulses) in the amygdala until three consecutive stage 5 seizures were exhibited. Once kindled, suppressant stimulation was applied to the dorsal hippocampus. Preliminary results indicate that suppressant stimulation delivered to the dorsal hippocampus simultaneously with amygdaloid kindling stimulation dramatically reduced seizure severity from stage 5 to stage 1 in 80% of the trials. Afterdischarge duration recorded from the amygdala and hippocampus was reduced during 60% of the trials. Suppressant stimulation delivered to the dorsal hippocampus 4-8 seconds after seizure onset potentiated seizure severity and amygdaloid afterdischarge duration.

459.13

IS THE DEEP PREPYRIFORM CORTEX A CRUCIAL FOREBRAIN SITE FOR KINDLING ? D. P. Cain, M. E. Corcoran, K. A. Desborough* and D.J. McKitrick*. Depts. of Psychology, U. of Western Ontario, London N6A 5C2 & U. of Victoria, Victoria, V8W 2V2 CANADA.

Recently published data suggest that a restricted site in the deep prepyriform cortex (DPC) may be a crucial site for epileptogenesis in rat brain. To test the applicability of this idea to the kindling model, we electrically kindled different groups of rats in or near the DPC, or in pyriform cortex or amygdala (AM). In different groups of rats we also 1) kindled the AM and then tested for transfer kindling to the ipsilateral DPC, 2) made radiofrequency lesions of the DPC prior to kindling the ipsilateral AM, and 3) injected carbachol into the DPC using a syringe pump and implanted chemitrodes.

The rates of kindling in DPC (8.1 ADs), immediately surrounding areas (8.3), and pyriform cortex (8.9) were indistinguishable, and were marginally faster (Ps=0.051-0.08) than kindling in AM (11.0). The DPC lesion group kindled after 8.7 ADs, which did not differ from the AM group. Picomolar quantities of carbachol failed to evoke epileptiform spiking, but nanomolar quantities usually evoked spiking. Transfer kindling occurred after 2.8 ADs, which is consistent with the strong similarity in kindling between DPC and AM. Thus, DPC kindles readily at a rate that is similar to that of surrounding forebrain tissue, and the integrity of DPC is not necessary for normal AM kindling. Supported by Grants from NSERC to D.P.C. and M.E.C.

459.14

INTERACTION OF SUBSTANTIA INNOMINATA AND PREPIRIFORM CORTEX IN GENERALIZED ELECTROGRAPHIC SEIZURES. R.S. McLachlan, F. Bihari* and A. Au*. Dept. Clin. Neuro. Sci. and Physiol., Univ. of West. Ont., London, Canada N6A 5C1

Stimulation of either the substantia innominata (SI) or prepiriform cortex (PPX) has been shown to induce generalized seizures. Both of these areas have widely dispersed efferents including to each other. The role of SI and PPX in generalized seizures was studied by lesioning each area respectively and stimulating the other in halothane anaesthetized Wistar rats. Since PPX has a lower seizure threshold than SI, it was postulated that SI seizures might spread via efferents to PPX and that lesioning of PPX might reduce seizures.

After a cortical penicillin spike focus was established in the ipsilateral hemisphere, unilateral bipolar stimulation of SI (0.2-0.8mA, 0.2ms, 40HZ) was carried out to induce generalized electrographic seizures. Lesioning of PPX (0.3mA, DC current x 3 minutes) had no effect on seizures induced by SI stimulation (7/7 animals). However, using the same methodology, seizures induced by electrical stimulation of PPX were abolished or decreased in duration and in amplitude after lesioning of SI (10/13 animals). Stimulation and lesion sites were histologically confirmed. Results suggest that generalized electrographic seizures from SI stimulation are not mediated via PPX but that a PPX-SI interaction does occur in seizures following PPX stimulation.

459.15

INTRA-AMYGDALOID INJECTION OF KAINIC ACID: AN ACUTE LIMBIC SEIZURE MODEL SIMILAR TO KINDLING. G.C. Yeh*, L.S. Chen, and J.O. McNamara. (SPON: S.J. Schiff). Duke Univ.& VA Med. Ctr.,Durham, NC. 27705.

Analysis of anatomical structures underling kindling is hampered by the time-consuming nature of this model. We therefore examined seizures induced by intra-amygdaloid microinjection of the convulsant, kainic acid. Behavior and EEG consequences were observed over 90 minutes. Kainic acid produced a dose-dependent (0.02-2 ug) increase in EEG and behavioral seizure intensity; the behavior mimicking that produced by electrical stimulation in kindled animals. Intra-peritoneal administration of the alpha-2 adrenergic antagonist,Idazoxan (0.5mg/kg) markedly increased the seizure response to low dose (1 ug) kainic acid. Intra-nigral muscimol (50 ng/SN) attenuated the seizure response to high dose (2 ug) kainic acid. Microinjection of low dose (0.2 ug) kainic acid in animals previously kindled by intra-amygdaloid electrical stimulation evoked intense behavioral seizure mimicking high dose kainic acid.

Together these results indicate a marked similarity in the EEG and behavioral seizures evoked by chemical and electrical stimulation of the amygdala. Both seizures are controlled by substantia nigra and by an alpha-2 antagonist. Intra-amygdaloid injection of kainic acid may aid in elucidating the anatomical framework underlying kindling.

459.16

ACTIVE CONDUCTANCES IN BASOLATERAL AMYGDALA (BLA) NEURONS ARE AFFECTED BY AMYGDALA KINDLING. P. Shinnick-Gallagher, P.W. Gean* and A.C. Anderson*. Department of Pharmacology, University of Texas Medical Branch, Galveston, TX 77550.

This study was designed to analyze changes in the electroresponsiveness of BLA neurons resulting from kindling in the BLA. Kindling was produced by applying a subconvulsive stimulus, daily, through an electrode in the BLA. Contralateral brain slices were prepared 4-6 wks after the last stage 5 seizure. In control BLA neurons, an action potential elicited with a 5 msec cathodal pulse is comprised of a fast afterhyperpolarization (f-AHP) followed by depolarizing afterpotential (DAP), a component of which is a low threshold calcium spike (lts). The f-AHP in kindled neurons (maximum value: -59.7 ± 0.9 mV; n=31) was more negative than in control (-53.1 ± 0.8 mV; n=40). The enhanced f-AHP was accompanied by an apparent loss or depression of the DAP (or lts). Rebound lts's following hyperpolarizing prepulses observed in control were not recorded in the majority of kindled BLA neurons. Control BLA neurons fire 5 to 6 spikes during 500 msec cathodal pulses whereas kindled neurons do not accommodate. In kindled neurons, the amplitude of the slow-AHP following a cathodal pulse was significantly reduced 50% and the fall-time shortened 50%. The medium-AHP was not significantly different in kindled neurones. The data suggest that calcium and potassium conductances that control cell excitability may be permanently altered in kindled BLA neurons. (Supported by NS24643)

459.17

EFFECTS OF SMALL DOSES OF KETAMINE ON SEIZURE BEHAVIOR AND 2-DEOXYGLUCOSE UPTAKE INDUCED BY AMYGDALOID STIMULATION. L.E. White*, K.M. Carnes and J.L. Price. Dept. of Anatomy & Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

As previously reported, graded levels of electrical stimulation of the amygdala in awake, unrestrained rats produce graded seizure activity, as manifested by behavioral effects and 2-deoxyglucose (2DG) uptake (Carnes and Price, Neurosci. Abs. 13: 125.4, 1987). Low levels of stimulation, which did not produce generalized convulsions, resulted in a pattern of 2DG uptake that reflected the majority of known primary projections of the amygdala. Higher levels of stimulation produced convulsive behaviors (e.g. "wet-dog shakes", rearing and forelimb clonus) as well as more widespread, often bilateral patterns of 2DG uptake.

Low levels of ketamine (1 to 10 mg/kg) were adminstered intravenously, in an attempt to reduce generalized convulsions, while still using high levels of stimulation to evoke widespread 2DG uptake. The effect of ketamine was to reduce both convulsive behaviors and 2DG uptake, in a dose-dependent fashion. Smaller doses of ketamine (1 to 3 mg/kg) moderated the expected pattern of 2DG uptake and reduced the intensity of the behavioral effects. Higher doses of ketamine (5 to 10 mg/kg) restricted the pattern of 2DG uptake to the heaviest primary amygdaloid targets, and prevented all but the mildest behavioral effects.

Supported by NIH AGO5691 and NIH NS09518. KMC is also supported by GM07200, Medical Scientist, LEW by Washington University Graduate Fellowship.

459.18

AMYGDALA KINDLED SEIZURES PRODUCE DEFICITS IN ASSOCIATIVE LEARNING WHICH DIFFER FROM THOSE PRODUCED BY PENTYLENETETRAZOL-INDUCED SEIZURES. D.B. Peele and M.E. Gilbert. Northrop Environmental Sciences, RTP, NC 27709.

The effect of seizure activity on cognitive function was examined using the long-delay flavor-aversion paradigm in rats. Previous research has demonstrated a disruption of lithium-induced flavor-aversion conditioning with pentylenetetrazol (PTZ) administered prior to lithium administration (Shaw and Webster, Psychopharm., 66:195-198, 1979). The present experiment was designed to determine whether this disruption was due associative (delay-independent) or menmonic (delay-dependent) processes, and whether the reported disruption represents a functional consequence of all (i.e., chemically- vs electrically-induced) seizure activity. Saline treated rats receiving injections of lithium (0.9mEq) after consuming saccharin flavored water later avoided saccharin ingestion: the degree of avoidance varied inversely with the time (0.5 or 6 hr) separating initial saccharin availability and lithium injection. PTZ (50 mg/kg ip), administered just prior to lithium administration, impaired conditioning at the long but not the short delays, suggesting a mnemonic deficit. Amygdala kindled seizures, elicited 2-3 min prior to lithium produced a disruption of conditioning at both the short and long delays, suggesting a more global, associative impairment. These results suggest a dissociation of the functional consequences of chemically and electrically elicited seizure activity.

459.19

BRAIN STEM MECHANISMS OF SEIZURE SUPPRESSION IN REM SLEEP.M.N. Shouse*, J.M. Siegel, and A.R. Morrison (SPON: M.B. Sterman). VA Med. Ctr., Sepulveda, CA 91343; U. of Penn. Sch. of Veterinary Med., Philadelphia, PA 19104.

The antiepileptic properties of rapid-eye-movement sleep (REMS) are well known in humans, but the mechanism is obscure. This study showed that feline epilepsy models display the same resistance to generalized EEG paroxysms and tonic-clonic convulsions (GTCs) during REMS as humans and also dissociated the REMS components responsible for these effects. Bilateral electrolytic lesions of the medial pontine tegmentum produced a syndrome of REMS without atonia. IP injections of atropine (1.5 mg/kg) produced REMS with EEG synchrony. 32 cats (12 amygdala kindled, 12 systemic penicillin epilepsy, 8 ECS) displayed minimal GTC & penicillin spike-wave (SW) activity during REMS, when compared to other sleep-waking states, before lesions or atropine. Selective loss of atonia in REMS abolished REMS protection against ECS or kindled GTCs without affecting penicillin SW. Conversely, loss of EEG desynchronization in REMS abolished REMS protection against SW without influencing GTC threshold. These results indicate that the descending brain stem pathways which mediate lower motor neuron inhibition also protect against generalized motor seizures during REMS. Protection against spread of EEG paroxysms is governed by a separate REMS mechanism, presumably the ascending brain stem pathways mediating intense thalamocortical EEG desynchronization during REMS.

460.1

AGE-RELATED MORTALITY AND MORBIDITY DIFFERENCES FOLLOWING BRAIN INJURY IN THE RAT. R.J. Hamm, B.G. Lyeth, S.E. Robinson, M. Ray, L. Doane*, L.W. Jenkins*, G.L. Clifton, & R.L. Hayes. Richard Roland Reynolds Neurosurgical Research Laboratory. Medical College of Virginia,Richmond, VA 23298.

Age is a major independent factor affecting head-injured patients; increasing age is associated with higher mortality and morbidity (J.Neurosurg.,68:409,1988). We examined age effects on mortality and behavior after traumatic brain injury in rats.

Three-month-old (young) and 20-month-old (aged) Fischer 344 rats were anesthetized with Metofane and injured at mild (1.75-1.85 atm) or moderate (2.0-2.3 atm) magnitudes of fluid percussion brain injury (J.Neurosurg., 67:110,1987). Following injury, the acute duration of reflex suppression (e.g., pinna, corneal, righting, tail flexion) and long-term motor deficits (beam-walking) were recorded.

Mild injury produced mortality rates of 50% in aged and 25% in young rats. Moderate injury produced mortality rates of 100% in aged and 20% in young rats. Recovery of reflexes was faster for young rats. Aged rats exhibited greater and longer lasting motor deficits. Our findings suggest that fluid percussion injury to the rat may be a useful model for the experimental study of aging effects on brain injury.

Supported by NIH Grant R01-NS-21458.

460.2

REDUCED URIDINE CONCENTRATION IN CSF AFTER I.C.V. BLOOD INJECTION. R.A. Mueller, T.J. McCown, R.D. Hunt,* and G.R. Breese. Departs. of Anesthesiology, Pharmacology, and Psychiatry, UNC-CH, Sch. of Med., Chapel Hill, NC 27599-7010.

Uridine in ventriculostomy cerebral spinal fluid (CSF) from adult head injury patients is less than 10% of normal.

CSF from male or female Sprague-Dawley rats was removed from the cisterna magna under light ether anesthesia at various times after neurological insults. CSF uridine, hypoxanthine and xanthine were measured by high pressure liquid chromatography.

Sublethal degrees of hypoglycemia, cerebral hypoxia or ischemia did not alter CSF uridine, but postmortem CSF uridine was unformly elevated. Injection of 200 of autologous non-heparinized blood into the lateral ventricle of rats after withdrawal of 75-100 CSF (for measurement of pre-injection CSF uridine) produced significant depression of cisternal CSF uridine 24 hrs. later, which gradually returned to normal over the next 48 hours. No similar changes were noted after serum or saline i.c.v. injection. All animals were neurologically intact after the ether effect dissipated.

It is possible that deceased uridine in head injury patients is the result of the presence of blood in the ventricular spaces. Since uridine is a potent endogenous anticonvulsant as well as an essential substrate, the sequelae of ventricular hemorrhage may be partly due to this metabolic disturbance.

460.3

TRAUMATIC BRAIN INJURY REDUCES QNB BINDING TO MUSCARINIC RECEPTORS IN RAT HIPPOCAMPUS. L.D. OLENIAK,* B.G. LYETH, T.J. MARTIN*, B.R. MARTIN, L.W. JENKINS, G.L. CLIFTON*, H.F. YOUNG* AND R.L. HAYES. Richard Roland Reynolds Neurosurgical Research Laboratories, Division of Neurosurgery, Medical College of Virginia, Richmond,VA 23298.

Our laboratories have shown muscarinic agonist-receptor interactions may contribute to the pathophysiology of traumatic brain injury (TBI) (Brain Res., 448:88,1988). One component of the pathophysiology of TBI is the characterization of abnormal neuronal information flow which may be reflected by changes in receptor binding. The present study examined muscarinic receptor binding following TBI. Male Sprague-Dawley rats (280-340g) were either sham injured or given moderate fluid percussion brain injury (2.25-2.35 atm) (J. Neurosurg., 67:110,1987). Animals were anesthetized with methoxyflurane and immersed in liquid N_2 at 5min, 3hrs and 24hrs after injury. Ten micron brain sections were mounted on subbed slides, incubated in .5nM [^{3}H]-QNB, air dried and placed against ^{3}H-Hyperfilm for 21 days. Binding was quantified using computer assisted image analysis. Preliminary data show a 30-40% decrease in binding as compared to control in the dentate gyrus and CA_1 regions of the hippocampus at 3hrs post injury. Overstimulation of muscarinic receptors may cause decreases in receptor binding. These data suggest that abnormal muscarinic agonist-receptor interactions occur following TBI. Decreased binding may possibly result from activation of intracellular second messenger systems. Supported by NIH Grant R01-NS-21458.

460.4

EFFECT OF MANNITOL-INDUCED HYPEROSMOLALITY ON BRAIN INTERSTITIAL FLUID TRANSPORT. G.A. Rosenberg, J. Barrett*, E. Estrada*, W. T. Kyner*. Neurology Service, VA Medical Center and Department of Neurology, UNM, Alb., NM 87131

Mannitol is used to treat brain edema, but its mechanism of action is controversial. Therefore, we studied the effect of mannitol-induced hyperosmolality on brain interstitial fluid (ISF) by autoradiography. Adult cats underwent intracerebral infusion of the extracellular marker, ^{14}C-sucrose. Nine animals were given 2 gms/kg of mannitol intravenously, and another 9 animals without mannitol were controls. Plasma and CSF osmolality were measured. After 2 hours the brains were removed for determination of water and electrolyte content and for preparation of the autoradiograms. Diffusion coefficients were calculated for intracerebral transport with equations for radial diffusion. Mannitol increased plasma osmolality and lowered water and potassium contents in the white matter. Diffusion was reduced in the direction of gray matter into the white matter. We conclude that low doses of mannitol control CSF pressure by selectively removing water from white matter, reducing CSF volume, and affecting molecular transport at the gray/white interface.

460.5

NEURAL NETWORK COMBINATORICS: IMPLICATION FOR RECOVERY FROM BRAIN DAMAGE. R. B. Glassman. Dept. of Psychology, Lake Forest College, Lake Forest, IL 60045.

The factors determining whether a neuron in a particular set is active include its threshold and how many connections converge onto it from neurons in another set. Consider a simplified general case of a hypothetical set of N neurons, which constitute a source of input. Each possible combination of M of these N neurons is connected to a specialized neuron in a receiving set of feature analyzers ("fa's"). The threshold of each fa is such that it will be fired if any r of its inputs are active. At a given time, a of its inputs are active. (Note that $N \geq M \geq a \geq r$.) Then the total number of fa's active is

$$\sum_{k=0}^{a-r} \left[({}_aC_{(r+k)}) \, ({}_{(N-a)}C_{(M-r-k)}) \right]$$

This formula, and more complex variants of it, have implications for restricted sizes of neuronal pools and numerosity of connections among pools. (As r decreases, the network shifts from AND- to OR-type operation.) The formulas also provide a way to estimate reliability and to estimate the sacrifice of discrimination associated with arousal or with threshold adjustments compensating for the shock or "diaschisis" component of a deficit that follows brain damage.

460.6

TIME-INTENSITY RECIPROCITY FOR MICROWAVE EXPOSURE OF MICE IN VIVO OVER EXTENDED TIME PERIODS. B. Pulford* and M.W. Luttges. Engineering Sciences, University of Colorado, Boulder, Colorado 80309.

This study is an attempt to assess the effects on the nervous system of mice of different time-intensity combinations of microwave radiation. Current microwave exposure standards in the U.S. are not time dependent, whereas many other countries do incorporate a time factor into their standards.

Carbon loaded teflon cortical screws were implanted in HS mice one week prior to exposure. Experimental mice were placed in a restraint located at an H field maximum of an S band waveguide. This precision of placement within the field allowed us to determine mid-brain SAR's accurately. "Yolked" control mice were similarly restrained and placed in a sham waveguide. The microwave signal was 2450 MHz pulsed and the specific absorption rates(SAR's) used were 1.0 and 10 mW/g. Spontaneous electrocorticograms(ECoG's) and evoked responses to a strobe light stimulus were recorded from exposed and control mice at specific times during the 8 hour recording period. The exposed animals showed a decrease in total ECoG spectral power(10-50Hz) and a depression in the amplitude of the evoked responses. In both indices, a short term(<30min) large, time-intensity reciprocity was recorded as well as a smaller, long term reciprocity. The results indicate that exposure standards need to be based on a reciprocal relation between time and intensity of microwave radiation. Further detailed studies should specify the nature of such a standard.

460.7

EFFECTS OF METHYLPHENIDATE ON RECOVERY FROM ABLATION-INDUCED HEMIPLEGIA. A.E. Kline*, T.P. Flores*, D.Y. Tso-Olivas*, M.J. Chen, and D.M. Feeney. Depts. of Psychol. and Physiol., UNM, Albuquerque, New Mexico 87131.

A single administration of d-Amphetamine (AMPH) given to rats 24 hrs after sensorimotor cortex ablation (see CRC Critical Reviews in Neurobiol., 3(2):135-197, 1987) combined with relevant experience during the period of intoxication produces an enduring acceleration of recovery of locomotion. This study tested methylphenidate (MPD) in a rat model of hemiplegia. After training rats to run a narrow elevated beam, a right sensorimotor cortex ablation or sham surgery was performed. Twenty-four hr after surgery, 3,6,8,9,10,12, or 15 mg/kg of MPD or vehicle was given (i.p.). Beam-walk testing resumed at 1,2,3, and 6 hr post-injection and every other day for 15 days. No significant effects of a single dose of MPD was obtained when compared to saline controls. The reported beneficial effects of MPD on cognitive function after brain injury (J. Clin. Exp. Neuropsychol., 9(3):297-309, 1987) may require continuous medication. Alternatively, this rat hemplegia model is inadequate for screening drugs affecting cognitive deficits. Supported by DHHS Grant NS20220-02 and by MBRS 2S06 RR08139-14.

460.8

ALPHA TOCOPHEROL-LIPOSOMES FACILITATE RECOVERY FROM FRONTAL CORTEX LESIONS IN THE RAT. S. W. Hoffman*, M. Halks-Miller, and D. G. Stein. Clark University, Worcester, MA 01610 and SRI International, Menlo Park, CA 94025.

Twenty-four male Sprague-Dawley rats approximately ninety days of age received either bilateral medial-frontal cortical lesions or sham operations. Subjects received liposomes (SRI International) with or without physiologic amounts of d-alpha tocopherol (αT) (Kodak). Following the injury, 0.1 cc of the treatment material was flushed directly into the wound cavity. The treatment was delivered directly to the lesion site by PE60 tubing attached to subcutaneously implanted Alza Alzet pumps (Model 2001). After seven days of continuous treatment, the pumps were removed and subjects were rested for seven days. Subsequently, rats were trained on a delayed-spatial alternation task for a water reward. The results of this study showed that, in comparison to sham operates, brain-injured subjects given liposomes alone were significantly impaired on this spatial task. However, rats with lesions treated with αT-containing liposomes took significantly fewer days and made fewer errors than lesion controls in attaining 9 out of 10 correct responses. Histological analyses will be described. This research is supported by NIMH Grant No. 9R01NS25685.

460.9

EXACERBATION OF TRAUMATIC BRAIN INJURY FOLLOWING CENTRAL ADMINISTRATION OF KAPPA-OPIATE RECEPTOR AGONISTS. T.K. McIntosh, R. Romhanyi*, I. Yamakami*, A.I. Faden. Dept. Surgery, Univ. of Conn. Health Ctr., Farmington, CT 06032 and Dept. Neurology, Univ. of Calif., San Francisco 94143.

We have previously suggested that endogenous κ-opioid receptor systems may be involved in the pathophysiology of traumatic brain injury. The present study examined the effect of centrally administered κ-opiate agonists dynorphin 1-17 and U50,488H on neurological outcome after fluid-percussion (FP) traumatic brain injury (2.5 atmospheres) in anesthetized male, Sprague-Dawley rats (350-450g, n=40). At 15 min postinjury, animals randomly received an intracerebroventricular (ICV) injection of either 1) dynorphin 1-17 (30nM, n=8), 2) dynorphin 2-17 (des-tyr dynorphin, inactive at opiate receptors, 30nM, n=8), 3) U-50,488H (30nM, n=8), 4) U-50,488H (100nM, n=8), or 5) artificial CSF (equal volume=5μl, n=8). Dynorphin 1-17 and U-50,488H (100nM), but not dynorphin 2-17, U-50,488H (30nM) or CSF, significantly worsened neurological outcome and increased mortality after brain trauma. Dynorphin 1-17 but not CSF exacerbated the fall in cerebral blood flow in injured cortex, measured at 1 h post-trauma using radiolabeled microspheres. These data are consistent with the hypothesis that endogenous κ-opiate receptor systems mediate certain pathophysiological sequelae of traumatic brain injury.
(Supported by VA Merit Review 74R).

460.10

COGNITIVE, EMOTIONAL AND SOCIAL CHANGES IN ACUTE AND CHRONIC TRAUMATICALLY BRAIN INJURED: IT'S IMPLICATION FOR TREATMENT AND PREDICTION. D. Badillo-Martinez and M. Nelson*. DATAHR Rehabilitation Agency, Brookfield, CT 06804.

Frontal Lobe dysfunctions, new-learning problems and personality changes are frequent sequelaes to Traumatic Brain Injury (TBI). Reportedly these interfere with vocational rehabilitation efforts and return to pre-morbid functions. No systematic assessment of the concomitant impact of rehabilitation on cognitive, social and emotional functions within the same individual is reported. Moreover, the capacity for recovery is said to decline with chronicity. The cognitive-neuropsychological, emotional and social performance of two groups of severe TBI survivors, acute (1-3 years) and chronic (5 years +), were assessed at two intervals while participating in the multiple rehabilitation procedures. Findings compare the longitudinal changes in cognition, social and emotional performance within each group and between acute and chronic TBI's. Issues of critical time for rehabilitation intervention, treatment efficiency, capacity for integration and reorganization are raised.

460.11

THE BLOOD-NERVE BARRIER, NOREPINEPHRINE, PROSTACYCLIN AND MALONDIALDEHYDE IN VASCULARIZED AND NON-VASCULARIZED NERVE GRAFTS. M. Shupeck*, K. K. Ward*, J. D. Schmelzer*, P. A. Low. Neurophysiology Laboratory, Department of Neurology, Mayo Foundation, Rochester, MN 55905 USA

We compared the efficacy of vascularized (VASC) and conventional (CONV) sciatic nerve grafts in restoring nerve conduction, the blood-nerve barrier, norepinephrine (NE) and 6-keto Prostaglandin $F_{1\alpha}$ (6KPGF; the stable prostacyclin metabolite) in the rat. We also measured malondialdehyde (MDA) content. There was a statistically non-significant increase in nerve action potential amplitude in the grafted segments of vascularized nerves at 1 and 2 months post-graft. The ^{14}C-sucrose PA product was increased in both CONV and VASC at 1 and 3 mo. and not different to each other. NE and 6-KPGF, the major vasoconstrictor and dilator of nerve microvessels were better restored in VASC than CONV reaching statistical significance for 6KPGF ($p<0.001$). MDA used as an index of oxygen free radical generation was not significantly different in the 3 groups.

Group	NE (pg/mg)	6KPGF (pg/mg prot)	MDA (ng/mg) dry wt	PA (10^{-5}/sec/g) 1 mo	3 mo
CTL	2.6±0.18	8.3±0.59	5.8±0.45		
VASC	2.1±0.26	6.7±0.49	6.6±1.02	11.6±1.5	11.8±1.9
CONV	1.9±0.37	4.0±0.32	6.9±0.77	9.6±1.2	9.6±1.7

The better restoration of 6KPGF and perhaps NE suggest that vascularized grafts may be more effective in restoring vasoreactivity of peripheral nerve following graft.

460.12

PENETRATING HEAD INJURY: A RODENT MODEL OF TRAUMATIC HEMIPLEGIA. A.S. Merians, A.W. Deckel. University of Medicine and Dentistry of New Jersey, Newark, N.J. 07107.

We have developed a rodent model of unilateral penetrating brain injury manifesting persistant long term deficits, specifically sensory and motor dysfunction similar to hemiparetic characteristics evident in head injured patients. Previous studies indicated that at 1 and 3 weeks the lesioned group demonstrated decreased reaction to tactile stimulation contralateral to the lesion, decreased balance and decreased spontaneous locomotor activity. In this study the course of recovery was extended to 6 weeks.

Twenty three male rats were anesthetized with sodium pentobarbital. After administering a craniotomy 4mm in diameter over the forelimb/hindlimb representation of the motor cortex, a metal baton was inserted into a guide tube and dropped on to the dural surface. The control(10) animals received the craniotomy but not the impact.

At 6 weeks some recovery was evident, however the lesioned (13) animals demonstrated persistent deficits in vertical motor activity, tactile sensation and balance. Histological results demonstrated prominant cavitation 5mm by 3mm.

This model of traumatic hemiplegia allows for future experimentation with acute and long term treatment strategies.

460.13

PRETREATMENT WITH A-4 REDUCES BEHAVIORAL DEFICITS FOLLOWING TRAUMATIC BRAIN INJURY (TBI) IN THE RAT. S.E. Robinson, R.M. Martin*, T.R. Davis* and E.K. Enters*. Dept. Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.

Activation of central cholinergic neurons has been implicated in the production of a flaccid comatose state following concussive brain injury (Saija et al., Brain Res., in press). In order to further prove the role of central cholinergic neurotransmission in the production of behavioral deficits following TBI, we studied the effect of A-4, a hemicholium-3 derivative which crosses the blood-brain barrier. Male Sprague-Dawley rats weighing 270-310 g (n=10 per group) were surgically prepared under pentobarbital anesthesia 24 h prior to injury by placing a hollow tube epidurally over a central craniotomy and were injected i.p. with either saline or A-4 (40 mg/kg) 1 h prior to TBI. This dose has previously been demonstrated to deplete central acetylcholine stores without affecting other neurotransmitter systems (Tedford et al., JPET, 240: 476, 1987). The rats received fluid percussion injury (2.2 atm) while under methoxyflurane anesthesia and were ventilated as necessary following injury. The duration of suppression of several reflexes (pinna, corneal, paw flexion, tail pinch and flexion, and righting) and responses (escape, head support, and spontaneous locomotion) were measured up to 60 min after trauma. A-4 significantly reduced the time to recovery of the corneal and righting reflexes and head support. This study lends support to the role of central cholinergic neurons in the production of transient behavioral deficits following TBI. [NS#24413 and NS#7288].

460.14

ENDOGENOUS PEROXIDATIC ACTIVITY AFTER SPINAL CORD INJURY. J.A. Ellison*, Selina C. Cortez*, and L.J. Noble. Dept. of Neurology, School of Med., UCSF, San Francisco, CA 94122.

In order to identify endogenous peroxidatic activity (EPA) after spinal cord injury, sections of cord from 0.5 to 2 cm from the injury were reacted in diaminobenzidine (DAB) in the presence of H_2O_2. At 3 h after injury reaction product (RP) was restricted to hemorrhagic areas in the dorsal column, and appeared to be primarily related to peroxidatic-like activity associated with hemoglobin. At 1 and 2 wk RP was maximal at the boundary between gray matter and dorsal column and was present up to at least 2 cm from the injury. RP was mostly confined to large conglomerates of irregularly contoured cells whose cytoplasmic processes extended into the neuropil. Frequently, these processes were closely associated with blood vessels, an observation confirmed at the ultrastructural level. Preliminary observations of sections reacted with anti-glial fibrillary acidic protein suggest that certain of these cells expressing EPA may be astrocytes.

To determine the nature of EPA, tissue was incubated with one of the following: DAB and H_2O_2; DAB; H_2O_2; 2,6 dichloro-phenolindophenol (DCPIP, a catalase inhibitor); 3,amino 1,2,4 triazole (AT, a catalase inhibitor); or potassium cyanide (KCN, a hemeprotein enzyme inhibitor). No EPA was noted with omission of either DAB or H_2O_2. KCN inhibited EPA, however, both AT and DCPIP did not.

(Supported by NIH NINCDS RO1NS23324 to L.J. Noble).

460.15

MEMBRANE LIPID CHANGES CORRELATE WITH DECREASE IN TISSUE MAGNESIUM FOLLOWING IMPACT SPINAL CORD TRAUMA IN RABBITS. M. Lemke*, P. Demediuk* and A.I. Faden (SPON: T.P. Jacobs). Dept. of Neurology, University of California, V.A. Medical Center, San Francisco, CA 94121.

Secondary neurochemical events following traumatic spinal cord injury (SCI) appear to contribute to development of tissue damage and subsequent neurological deficit. We have shown that reductions in tissue Mg^{2+} following traumatic SCI in rats are significantly correlated with the injury severity. Membrane lipid hydrolysis and eicosanoid production occur after SCI in cats and rats. In the present studies changes in water content (wet/dry weight method), tissue Na^+, K^+, and Mg^{2+} (atomic absorption spectroscopy), phospholipids (HPLC) and free fatty acids (gas chromatography) were examined in the lumbar (L-2) segment of anesthetized rabbits following moderate (40 g-cm) and severe (150g-cm) impact injury. At 4 h posttrauma decreases in tissue Mg^{2+} and phospholipids were observed associated with elevation of arachidonic acid. These changes were significantly correlated with injury severity, whereas alterations in water content, Na^+, and K^+ were not. Thus, membrane lipid hydrolysis may lead to decreased binding sites for Mg^{2+}, resulting in a reduction of tissue Mg^{2+}. Given the critical regulatory role of Mg^{2+} for essential cell functions including glycolysis, oxidative phosphorylation and receptor activity, Mg^{2+} changes after trauma may play a fundamental role in secondary tissue damage.

460.16

REGIONAL CHANGES IN GLUCOSE AND ATP CONTENTS FOLLOWING EXPERIMENTAL SPINAL CORD COMPRESSION TRAUMA IN THE RAT A.C. Nacimiento, G. Adler* and A. Mautes*
Neurosurgical Research Laboratory, Saarland University Medical School, 6650 Homburg/Saar, F.R.G.

To study regional metabolic aspects of acute posttraumatic changes in the spinal cord following compression in the model of Nacimiento et al. (J. Neurosurg., 1985, 62, 898) we applied a bioluminiscence technique described for brain tissue by Paschen et al. (J. Cereb. Bl. Flow Metabol., 1985, 5, 465). Correlation between computer-assisted measurements of optical densities in bioluminiscent images of glucose and ATP distribution in single spinal cord sections, and contents in corresponding tissue samples was highly significant. Thus, both metabolites could be quantitatively determined by image processing of the bioluminiscent sections. Two hours after compressing 60 % of dorsoventral extent of segment L4 for 50 ms, we found significant elevation in glucose and reduction of ATP content in L4 and L6 segments. Changes in L2 were not significant. This method disclosed regional differences in posttraumatic carbohydrate and energy metabolism which cannot be resolved by measurements in tissue samples.

460.17

TRAUMATIC BRAIN INJURY PRODUCES TRANSIENT REDUCTION IN BINDING TO EXCITATORY AMINO ACID RECEPTOR SUBTYPES. R.L.Hayes, L.P.Miller*, B.G.Lyeth, L.W. Jenkins*, L. Oleniak*, G.L. Clifton* and H.F. Young*. Dept of Surgery, Medical College of Virginia, Richmond, VA 23298, Vet Adm Med Ctr, Wash D.C. 20422 & Dept of Pharm, Georgetown Univ Sch. of Med.,Wash. D.C. 20007.

Abnormal agonist-receptor interactions may contribute to the pathophysiology of traumatic brain injury (TBI). Recent data have demonstrated that blockade of muscarinic (Br Res 448:88,1988) or N-methyl-D-Aspartate (NMDA) (Neurosci Abst 13:1254, 1987) receptors can attenuate behavioral deficits associated with TBI in the rat. In the present report we have examined temporal changes in excitatory amino acid (EAA) receptor subtypes following fluid percussion TBI in the rat. EAA receptors were characterized by NMDA-displaceable 3H-Glutamate binding to the NMDA receptor, 3H-AMPA binding to the quisqualate receptor, and 3H-Kainic acid (KA) to the KA receptor. Our results show that NMDA receptor binding in the strata radiatum of the hippocampus decreased to 83% of control within 3 hr post-TBI and remained significantly below control levels for up to 24 hr (90% of control). Other regions such as the dentate gyrus, outer and inner layers of the cortex showed a somewhat similar pattern for NMDA binding. 3H-AMPA binding while not significant at any time point showed a pattern toward decreased binding at both 5 min and 3 hr post-TBI, while returing to control levels by 24 hrs. 3H-KA binding decreased significantly only at 24 hrs post-TBI and only in the inner layers of the cortex. Our results suggest that NMDA receptors apparently 'down regulate' in response to TBI. This response may be related to TBI-induced rise in extracellular glutamate concentrations. These results will also be discussed in relation to the TBI-induced vulnerability to subsequent ischemia and involvement of EAA receptors.

460.18

Experimental Obstructive Hydrocephalus: Sequential MRI's of rat brains over a 10-day time course suggests an age-related differential response in ability to resolve obstructive hydrocephalus. Kathryn A. Hager,*Juan A. Cabrera, M.D.* and Michael E. Miner, M.D., Ph.D., Division of Neurosurgery, University of Texas Medical School, Houston, TX, 77030.

In producing a reliable model of hydrocephalus in rats, the question of variation of age of the animal and effect this may have on the course of hydrocephalus has not been adequately addressed. It is the purpose of this study to begin to contrast these responses. Obstructive hydrocephalus was produced in 45 and 64 day old male, Sprague Dawley rats weighing approximately 150 g. and 250 g. respectively by injections of .05 cc sterile kaoline into the cisterna magna. Each animal brain was imaged via the utilization of MRI at 2,4,7, and 10 days post-operatively to follow the progression of ventricular dilation and resolution of hydrocephalus. The younger animals exhibited progressive ventricular dilation over the 10 day time course as well as continued physical deterioration. In contrast, the older group exhibited evidence of resolution of ventricular dilation in parallel with improvement of classical symptoms of hydrocephalus. Our findings indicate that the age-related differential response represents an important variable affecting the outcome in experimental obstructive hydrocephalus studies.

460.19

AXONAL LOSS AFTER GRADED SPINAL CORD CONTUSIVE INJURY IN THE RAT. J.R. Wrathall and L. Verma*. Dept. of Anat. and Cell Biol., Georgetown Univ., Washington, D.C. 20007.

We have previously examined the development of acute pathological changes after contusive injury in axons analyzed at the light and electron microscopic level. We used stereological techniques to develop a quantitative description of axonal changes in the ventro-medial white matter at 15 min, 2, 4, 24, and 48 hrs after a standardized mild contusive injury (*Soc. Neurosci. Abstr.* 13:1500,1987). We have now extended this study to include acute axonal pathology after severe contusive injury and to compare axons in acute versus chronic injury sites 8 weeks after mild contusion. The results indicate that the time course of the development of axonal pathologies in ventro-medial white matter is similar after severe or mild contusion. By 15 mins after injury there is a significant reduction in the volume ratio of normal axoplasm that is similar in mild and severe injuries. This is further reduced between 15 mins and 2 hrs after which it remains virtually constant through 8 weeks after contusion. Thus, in both mild and severe injury the significant axonal pathological alterations that lead to axonal loss appear to have been accomplished by 2 hrs after trauma. In addition, examination of the chronic injury sites at 8 weeks also indicate a preferential loss of the larger myelinated axons and increased myelin thickness for the remaining small axons as compared to axons of similar size in the normal spinal cord. (Supported by NIH-NO1-NS-2-2310)

460.20

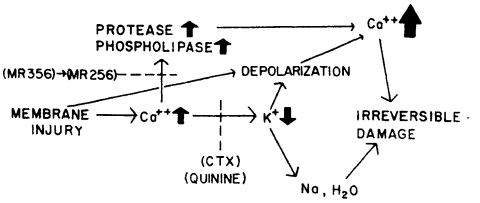
TWO NEW PHARMACOLOGIC AGENTS WHICH PROTECT THE RAT SPINAL CORD FROM CONTUSION INJURY. S. Tsuyoshi Ohnishi, Hiroaki Katagi* and Masayuki Katsuoka*. Membrane Research Institute, Phila. PA 19104.

Using the weight-drop technique (10g x 5cm), we studied the protecting effect of two new drugs in spinal cord injury of the rat. The efficacy was assessed by recovery of motor performance (Tarlov score) during 4 week period after injury.

(1) Charybdotoxin, a specific inhibitor of Ca-activated K efflux, demonstrated protective effect in pre-injury administration (0.12 mg/kg i.p.; 30 minutes before injury). Quinine also had a beneficial effect.

(2) Oligomeric derivatives of prostaglandin E_1 (6 mg/kg of either MR-256 or MR-356; i.p., post-injury administration), which may act as inhibitors for phospholipases and proteases, also had protective effect.

Possible mechanisms of protection are speculated.



460.21

INCREASE IN EXTRACELLULAR GLUTAMATE AND ASSOCIATED MASSIVE IONIC FLUXES FOLLOWING CONCUSSIVE BRAIN INJURY. Y. Katayama, M.K. Cheung, L. Gorman, T. Tamura* and D.P. Becker. Div. of Neurosurgery, UCLA, Los Angeles, CA 90024

Using microdialysis, changes in extracellular concentration of amino acids and inorganic ions ($[K^+]_e$ and $[Ca^{2+}]_e$) following concussive (fluid-percussion) brain injury were determined in the rat hippocampus. At the moment of injury, the microdialysis probe (O.D. 300 u) was temporarily withdrawn. A large increase in $[K^+]_e$ (4-5 fold baseline) occurred following the injury which produced unconsciousness lasting for more than 3 min. Moderate but significant decrease in $[Ca^{2+}]_e$, preceded by initial transient increase, was also demonstrated. Concomitantly with the $[K^+]_e$ increase, an elevation of excitatory amino acids, especially glutamate, was noted (4-5 fold baseline). Such a large increase was not observed in other amino acids. The $[K^+]_e$ increase and the $[Ca^{2+}]_e$ decrease were resistant to *in situ* administration of tetrodotoxin (10^{-4} M) through the microdialysis probe but effectively attenuated by kynurenic acid (10^{-2}M), a glutamate antagonist. These results suggest that a release of excitatory amino acids underlies the massive ionic fluxes following concussive brain injury.

460.22

ULTRA-RAPID CRYO-JET FREEZING OF MOUSE SPINAL CORD EXPLANTS. W.B. Greene* and J.D. Balentine. Dept. of Pathology and Laboratory Medicine. Medical Univ. of SC, Charleston, SC 29425.

Cryo-fixation is essential for the preservation of diffusible ions in preparation for x-ray microanalysis. Cryo-slamming has been used in our laboratory to prepare spinal cord explants. Cryo-jetting was developed for the difficult task of freezing rat spinal cord *in vivo* (Greene, W.B. & Balentine, J.D., abstract in press). The present study compared cryo-jetting with cryo-slamming of the *in vitro* spinal cord explants for a parallel *in vivo* & *in vitro* spinal cord trauma investigation. Control & traumatized explants, using techniques previously described (Balentine, J.D., Greene, W.B. & Bornstein, M., Lab. Invest. 58:93, 1988) were subjected to either slam-freezing using a Heuser type system with liquid N2 at -195°C or to jet freezing with liquid propane at -190°C and 150psi pressure. After cryo-fixation and freeze substitution, blinded assessment of sections revealed better preservation with cryo-jetting in both control and traumatized explants. The latter experiment cultures revealed clearly distinguishable focal areas of edema and axonal swelling. Cryo-jetting is a preferred method of fixation in the systems employed. (NIH Grant NS11066).

460.23

EXOGENOUS LAMININ/ARA C PROMOTES "REPAIR" OF SPINAL CORD, J. Miller and M. Politis, (C. Fischette spon.), Ortho Surg., U.B.C., Vancouver V5Z 4H4

Exogenous laminin can support axon elongation in vivo. Administration of AraC (cytosine arabinofuranoside) after CNS injury inhibits reactive gliosis. In the present study, these approaches were combined after rat spinal cord injury.

Cord were subjected to contusion lesion at T8. Elvax pellets containing laminin were inserted subdurally over dorsal columns and AraC administered by daily i.p. injection. Rats were assessed behaviorally and morphologically for 21dpo.

Behavioral studies (including inclined plane test and assessment of gait and toe spread) indicative significant "recovery" when laminin pellets were used in combination with AraC, but not when either treatment was used alone. Results were corroborated by elongating axons in dorsal columns rostral to lesion sites in laminin/AraC rats. HRP tracer studies to date suggest growth of rubrospinal and lat. vest-spinal fibers caudal to lesion site. Electrophysiological studies are in progress.

Studies are underway to determine if laminin/AraC treatment can be used in combination with other intervensions. Studies to date indicate no synergistic effects when combined with exogenous D.C. current.

460.24

ATTENUATION OF POST-TRAUMATIC SPINAL CORD ISCHEMIA BY THE LIPID PEROXIDATION INHIBITOR U74006F: COMPARISON IN A CONTUSION VS. COMPRESSION MODEL. E.D. Hall, P.A. Yonkers* and J.M. Braughler. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The ability of the 21-aminosteroid lipid peroxidation inhibitor U74006F to attenuate the development of post-traumatic spinal cord ischemia was examined in pentobarbital anesthetized cats after either contusion (300 g-cm) or compression (180 g x 5 min.) L3 injury. Spinal cord white matter blood flow (SCBF) was measured prior to and for 4 hrs. after injury using H_2 clearance. Vehicle or drug was given as an i.v. bolus at 30 min. post-injury. Following contusion injury, there was a gradual decline in SCBF which reached -63.5% by 4 hrs. In cats receiving a 10 mg/kg i.v. dose of U74006F, the 4 hr. decrease was only 9.9% ($p<0.001$). However, lower doses (1 or 3 mg/kg) were completely ineffective. In the compression model, there was an initial hyper-perfusion (not seen in the contusion model) which abated by 30 min. followed by a decline to a level at 4 hrs. only slightly greater than that seen after contusion injury. In this model, U74006F was found to be a more potent antagonist with the 1 and 3 mg/kg doses effectively reducing the decrease in SCBF. These results point out differences between contusion and compression spinal injury and that the potency of agents as antagonists of post-traumatic ischemia is dependent upon the injury model.

460.25

PROSTHESISWARE: MICROCOMPUTER BASED COGNITIVE PROSTHETICS IN NEUROREHABILITATION. D.L. Chute and M. Hoag*. Neuropsychology Program and Software Development Group, Drexel University, Philadelphia, PA 19104.

As a prosthetic tool useful in neurorehabilitation, the microcomputer has a number of advantages that directly support cognitive functions and activities of daily living. New classes of software like HyperCard and MacLaboratory Controller are easily customized to provide "ecologically relevant" prosthetic support that does not presuppose any reorganization or restructuring of damaged neural tissue. We present case studies of traumatic brain injury patients for whom ProsthesisWare programs have been customized with the ready editing and rapid turn-around capability allowing multiple iterations and successive approximations to reach clinical goals. The speech prosthesis SpeakEasy and related programs illustrate the development model used, highlight issues in neuropsychological assessment and treatment, show features of software interface and design relevant to the head injured patient, and offer new directions for cognitive rehabilitation and the role of rehabilitation facilities. In spite of some limitations, the emergence of ProsthesisWare offers utilitarian prosthetic aids for the everyday activities of daily living for some people with cognitive impairments. This research conducted in part at Moss Rehabilitation Hospital, Philadelphia, was supported by grants from the Solon Foundation, Apple Computer Inc, Drexel University and the Ben Franklin Partnership and complies with guidelines for human subjects.

OPIATES, ENDORPHINS AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS IV

461.1

NALTREXONE (NTX)-PRECIPITATED OPIATE WITHDRAWAL INCREASES LOCUS CERULEUS (LC) SPONTANEOUS DISCHARGE AND DISRUPTS LC SENSORY RESPONSES. E.F. Aulisi, R.G. Wehby* and R.J. Valentino. Dept. of Pharmacology, George Washington Univ. Med. Ctr., Washington, D.C. 20037.

LC discharge during NTX-precipitated opiate withdrawal was recorded in halothane-anesthetized rats 7 days after s.c. implantation with morphine (MOR)-containing osmotic minipumps. I.C.V. MOR (1.0 μg) had no effect on LC spontaneous discharge rates in MOR-implanted rats (n = 7). Because this dose of MOR is greater than that required to completely inhibit LC discharge of opiate-naive anesthetized rats, the results verify the development of MOR tolerance in the rats. I.C.V. administration of 1.0 μg NTX increased LC spontaneous discharge 152 ± 39% (n = 7). NTX also altered LC discharge evoked by repeated sciatic nerve stimulation such that the evoked discharge was decreased while background discharge was increased. The net effect of NTX was to decrease the ratio of evoked-to-tonic LC discharge during a block of phasic sensory stimulation. This effect is similar to that produced by certain stressors. NTX did not affect LC discharge of rats implanted with saline-containing minipumps. The present results confirm previous reports of increased LC activity during antagonist-precipitated opiate withdrawal and extend these findings by characterizing effects on LC sensory-evoked discharge. Supported by NIH Grants DA 03695 and MH 40008.

461.2

A NOVEL DECREASE IN ADENYLATE CYCLASE ACTIVITY IN THE RAT LOCUS COERULEUS IN RESPONSE TO ACUTE MORPHINE OR CLONIDINE ADIMINISTRATION. D. Beitner*, R.S. Duman, and E.J. Nestler (SPON: S.J. Strada). Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Previously, we have demonstrated two effects of morphine on adenylate cyclase activity (AC) in the rat locus coeruleus (LC): inhibition of AC by acute in vitro treatment in isolated membranes, an effect also observed in all other brain regions examined, and increased levels of AC by chronic in vivo treatment, an effect specific to the LC. In this study, we have identified a third and novel action of morphine on AC: a stable decrease in AC by acute in vivo treatment, which is retained in isolated membranes in vitro.

Rats received acute in vivo morphine treatment, after which AC was assayed in isolated brain membranes. Such morphine treatment produced a dose-dependent decrease (20-25% maximal) in enzyme activity in the LC, but not in other brain regions examined. Co-administration of naltrexone, an opiate receptor antagonist, blocked morphine's ability to produce this effect, indicating that it is mediated via the specific activation of opiate receptors. The decrease in AC in the LC is not due to morphine being retained in tissue fractions: the opiate receptor antagonist naloxone failed to reverse the effect in isolated membranes, and opiate agonists produced the same level of acute enzyme inhibition in vitro in membranes prepared from LC of control and morphine-treated rats. Interestingly, clonidine, which produces effects on the LC similar to those of morphine, also induced a decrease in AC after acute in vivo treatment, whereas diazepam, which has different actions on the LC, failed to produce this effect.

This stable decrease in AC activity may be an early step in the sequence of events that lead to the development of opiate tolerance and/or dependence in the LC.

461.3

HALOPERIDOL-SENSITIVE, SELECTIVE ELECTROPHYSIOLOGICAL EFFECTS OF SIGMA LIGANDS ON NCB-20 CELLS. J.A. Bell*, C.E. Spivak, T.-P. Su and E.D. London (SPON: L. Sharpe). Neuropharmacology Lab., NIDA Addiction Res. Center, Baltimore, MD 21224.

Although specific sigma binding sites are present in brain, studies on the effects of sigma-directed ligands on neurons have been lacking. Largent et al. (Eur. J. Pharmacol., 124:183, 1986) showed that hybrid cells (mouse neuroblastoma - Chinese golden hamster brain) of the NCB-20 line show high affinity sigma binding with a concomitant lack of phencyclidine (PCP) binding sites. We have obtained intracellular recordings from these cells with micropipettes (30 Mohm), and have studied the effects of pressure ejection of drugs on them. Sigma ligands (100 µM), (+)-3PPP, (+)-pentazocine and (+)-N-allylnormetazocine, ejected from micropipettes (3 µM tip diameter) depolarized these cells by 10-15 mV. Ejection of these drugs at 10 µM concentration produced smaller and less consistent effects. The depolarizing effect of these drugs were blocked by a bath concentration of 10 µM haloperidol. Haloperidol (10 µM) did not block the depolarizing effects of serotonin or dopamine. Morphine, l-glutamate and tetracaine (100 µM) did not depolarize NCB-20 cells. The selective PCP ligand MK-801 (100 µM) had a small depolarizing effect that was blocked by haloperidol. We conclude that NCB-20 cells may be a good neuronal model for studying sigma effects.

461.4

MPV 1440, A HIGHLY - SELECTIVE ALPHA-2 AGONIST, PREVENTS OPIATE - INDUCED MUSCLE RIGIDITY. M.B. Weinger, I.S. Segal*, and M.M. Maze*. Depts. of Anesthesiology, Univ. of California, San Diego, Stanford University, and the San Diego and Palo Alto V. A. Medical Centers.

Several studies have shown that the selective alpha-2 adrenergic agonist, MPV1440 [**M**], produces marked analgesia, muscle flaccidity and decreased sympathetic activity. Because recent work suggested that adrenergic systems might play a role in opiate-induced muscle rigidity, we studied the effects of pretreatment with **M** on muscle tone in rats given the potent short-acting opiate alfentanil [**A**]. Wistar rats had 15 minutes of baseline hindlimb electromyographic (EMG) activity recorded and were then injected with either saline [SAL], MPV1441 (the inactive stereoisomer), **M** (in several doses), or **M** and the alpha-2 antagonist, idazoxan. 15 minutes later, all rats received **A** (0.5 mg/kg s.c.) and data were collected over for 60 minutes. Data were analyzed using ANOVA and Newman-Keuls tests. There were no differences between groups in weight or baseline EMG activity. In the SAL and MPV1441 groups, **A** resulted in a marked increase in hindlimb EMG activity and the behavioral manifestations of opiate rigidity. In contrast, **M** dose-dependently prevented **A**-induced rigidity. The **M** animals were flaccid, akinetic, and lacked a startle response. The addition of idazoxan resulted in an elimination of **M**'s blockade of **A**-induced rigidity. The results of this study clearly demonstrate that pretreatment with The antagonism by idazoxan of **M**'s prevention of **A**-induced muscle rigidity supports an alpha-2 mediation of this phenomenon. The **M** doses necessary to antagonize **A** rigidity are relatively small (<1/10) compared with doses used alone reported to produce an anesthetic state. The results of these studies support a role for alpha-2 adrenergic systems in the expression of opiate-induced muscle rigidity in the rat.

461.5

OPIATE-MEDIATED BEHAVIORS AND BRAIN OPIOID PEPTIDES IN NEONATES AFTER PRENATAL ETHANOL. P. Kehoe, E. Vavrousek-Jakuba and W.J. Shoemaker. Trinity College, Hartford, CT & U Conn. Health Center, Farmington, CT.

Pregnant rats were fed either an alcohol-containing diet, an isocaloric pair--fed diet or standard chow. At birth all offspring were fostered onto control mothers, eight per litter, and tested at ten days of age. Similar groups were sacrificed at 12 days of age for brain B-endorphin levels using a specific RIA. The ten-day-old rats were isolated and monitored for ultrasonic vocalizations after naltrexone (0.5mg/kg) administration. After 10 min isolation they were tested for analgesia to heat. Chow-fed and pair-fed saline controls vocalized 200 times during isolation which doubled with naltrexone pretreatment. Alcohol-treated pups, however, vocalized significantly less after saline (75) and naltrexone (160). Additionally, these pups did not demonstrate the isolation-induced analgesia seen in controls but did show a marked increased sensitivity to heat after naltrexone administration. Prior studies have demonstrated that prenatal ethanol results in markedly increased levels of B-endorphin at birth that persists to adulthood. Our current studies confirm the earlier findings and suggest that the unusual responses of the alcohol offspring in opiate-mediated behaviors may be due to a primary defect in B-endorphin levels or, secondarily, to abnormalities of the opiate receptors.

Supported by NIAAA #06927.

461.6

HETEROGENEITY IN ENDOGENOUS OPIOID CONTROL OF FETAL OVINE CARDIOVASCULAR FUNCTION. C.E. Dunlap and N.K. Valego*. Bowman Gray Sch. Med. of Wake Forest Univ., Winston-Salem, NC 27103.

Agonists for three opioid receptor subtypes, δ (leucine-enkephalin), κ (dynorphin1-13) and μ (morphine) were studied in 3 to 7 fetal sheep 100 to 124 days gestation.

Treatment	MAP (torr)	HR (bpm)
Intact Control	45.0±1.5	179±18
Intact, 0.5mg/kg LEK	35.3±2.6	99±24
Intact, 0.5mg/kg DYN	57.9±3.1	148±36
Vagotomy Control	39.5±2.6	167±14
Vagotomy, 0.5mg/kg LEK	41.0±4.9	190±36
Vagotomy, 0.5mg/kg DYN	62.5±1.8	266±46

Morphine had no effect on mean arterial pressure (MAP) or heart rate (HR) in doses up to 10 mg/kg. Leucine-enkephalin (LEK) produced a brief, significant decrease in MAP and HR, which was abolished by vagotomy, similar to effects previously reported for met-enkephalin (LaGamma et al., Pediatr Res 17:162-67, 1983). Dynorphin1-13 (DYN) produced a significant increase in MAP which lasted up to 45 min, and was not abolished by vagotomy. DYN had no effect on HR in intact fetuses, but produced a significant increase in HR in vagotomized animals. These results suggest differential control of CV function by at least two endogenous opioid systems in developing sheep. Research Supported by NIH Grant HL34460 to CED.

461.7

DISINHIBITION OF PITUITARY LH BY INTRACEREBRAL ANTI-OPIOID TREATMENTS IN SHEEP. G.D. Weesner* and P.V. Malven. Dept. Animal Sci., Purdue Univ., W. Lafayette, IN 47907.

This research sought to determine if β-endorphin (βE) exerts a physiological inhibition on LHRH release in the CNS of sheep. Nine mature ewes were implanted with guide tubes through which matched infusion cannulae could subsequently be briefly inserted for intracerebral (ic) infusion of naloxone (NAL; 50 μg in 20 μl), normal sheep serum (NSS; 20 μl of 1:25), or sheep anti-sheep βE (SASβE; 20 μl of 1:25; Ebling and Lincoln, Endo. 120:809). To detect abrupt disinhibition of LHRH release by anti-opioid treatments, serum LH was quantified at 10-min intervals for 1-2 h before and after each ic infusion. During the luteal phases of recurring estrous cycles, complete experiments (i.e., 3 different ic infusions at each site over 27 h period) were performed 2-4 times/site. LH assays and histological analyses of CNS sites showed that SASβE consistently disinhibited LH/LHRH release when infused at the following sites: (A) rostral preoptic area/nucleus accumbens (2 ewes) and (B) anterolateral hypothalamus (1 ewe). Infusion of NAL also disinhibited LH/LHRH release at site A but not at site B. In addition, NAL disinhibited LH/LHRH release at 5 other sites (3 in areas slightly rostral to site A and 2 in mediobasal hypothalamus), but serum LH responses to SASβE were not consistently different from those to NSS. Three other sites were not responsive to either NAL or SASβE. In summary, ic immunoneutralization of endogenous βE in selected CNS sites relieved the inhibition of LHRH release. However, other opioid ligands may also be physiologically important in luteal-phase sheep since the nonspecific anti-opioid NAL was effective at more sites than SASβE.

461.8

STIMULATION OF MESOLIMBIC AND MESOCORTICAL BUT NOT NIGRO-STRIATAL DOPAMINE RELEASE BY MORPHINE. P.L. Wood and T.S. Rao*. CNS Diseases Research, G.D. Searle & Co., c/o Monsanto Co., St. Louis, MO 63198.

Previous studies have demonstrated that morphine increases dopamine (DA) synthesis and metabolism in nigrostriatal, mesolimbic and mesocortical dopaminergic projections (Kim et al., Life Sciences 39:2033, 1986). However, the effects of morphine on regional DA release have not been evaluated to date. In this report we present data concerned with the effects of morphine on steady-state 3-methoxytyramine (3-MT) levels and on 3-MT accumulation after inhibition of monoamine oxidase, both indices of DA release. Morphine (16 mg/kg, sc; 60 min) was found to increase DA release in the rat olfactory tubercle, prefrontal cortex, pyriform cortex and N. accumbens. In contrast, DA release in the striatum was unchanged after treatment with morphine.

These data support previous observations that morphine acts to uncouple DA synthesis and release in the rat striatum but that this uncoupling is not present in other DA projections to cortical and limbic terminal fields.

461.9

AUGMENTATION OF MORPHINE-INDUCED CHANGES IN CEREBRAL MONOAMINE METABOLISM IN RATS TREATED CHRONICALLY WITH NALTREXONE. L.Ahtee* and K. R. Carlson. Dept. of Pharmacy, Univ. of Helsinki, SF-00170 Finland.

Chronic naltrexone treatment enhances the antinociceptive effect of morphine as well as the binding of opioid ligands to μ- and δ-sites (Tempel, A. et al., J. Pharmac. exp. Ther. 232: 439, 1985). To investigate the role of opioid receptors in the regulation of cerebral monoaminergic neurons we studied the effects of morphine (3-30 mg/kg, 2 h, s.c.) on the concentrations of DA, NA and 5HT and their metabolites in several brain areas of male rats one day after withdrawal from 14 day treatment with naltrexone (Alzet mini-osmotic pumps). Chronic naltrexone elevated the concentration of 5HT in the limbic forebrain, the hippocampus and the lower brain stem, that of NA in the lower brain stem, and decreased that of 3MT in the striatum. In naltrexone-treated rats morphine elevated the cerebral DOPAC, HVA, MHPG and 5HIAA concentrations as well as diminished the 3MT concentrations more or in smaller doses than in control rats. Thus the opioid receptors which mediate the opioid regulation of monoaminergic neurons seem to become supersensitive during chronic naltrexone treatment.

Supported by the Research Council for Medicine of the Academy of Finland (LA) and by USPHS Grant DA 02226 (KRC).

461.10

EVIDENCE FOR MU RECEPTOR AND 5-HT LINK IN EXPRESSION OF MORPHINE INDUCED HYPERACTIVITY. S. Gurtu. Department of Pharmacology, K.G.Medical College, Lucknow,226003,INDIA.

Opioid agonist morphine, in high doses, induces hyperactivity in rodents and other species. Intraperitoneal (ip) administration of morphine (30mg/kg) consistently evokes marked enhancement of spontaneous locomotor activity (SLA) in naive mice. This naloxone antagonizable, excitatory response is of an established central origin but the opioid receptor subtype involved in this action is yet to be distinctly recognized. We tested opioid agonists and agonist-antagonists for SLA potentiating effects in naive mice, using photoactometer. Pentazocine, a mu receptor antagonist and kappa and sigma agonist, did not evoke any increase in SLA even with doses upto 60 mg/kg, ip, while a relatively selective mu agonist, fentanyl, (0.5 mg/kg, ip) produced marked hypermotility. Prior administration of pentazocine (30 mg/kg, ip) blocked morphine as well as fentanyl induced potentiation of SLA. The excitatory effects of opioid agonists on SLA thus appear to originate from an activation of mu receptors. Additionally, the manifestation of these mu receptor mediated effects requires an intact serotonergic system since both morphine and fentanyl failed to augment SLA in mice in which serotonin had been depleted by prior administration of fenfluramine.

461.11

LESION OF THE NIGROSTRIATAL DOPAMINE PATHWAY INDUCES FUNCTIONAL DOWN REGULATION OF DELTA-OPIOID AND GABA RECEPTORS IN THE GLOBUS PALLIDUS. H.S. Pan and J.R. Walters. NINCDS, NIH, Bethesda, MD 20892.

In rats, 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway induces opioid and GABA receptor down regulation in the globus pallidus (GP, Pan et al., J. Neurochem., 1985). We used single unit recording techniques to examine the effects of 6-OHDA lesion on the sensitivity of GP neurons to microiontophoretically applied D-ala^2-D-leu^5-enkephalin (DADLE) and GABA 5-6 week post lesion in chloral hydrate anesthetized rats. Furthermore, the effects of chronic infusion of naloxone HCl (1.5 mg/day/rat for 2 weeks) 4 weeks after the induction of the lesion were studied. In normal rats, DADLE inhibited 45% of GP cells studied (n=29) > 80% baseline, 17% of cells > 50%, 10% of cells > 20%, and had no effects on 28% of cells. Naloxone (0.5 mg/kg, i.v.) reversed DADLE's effects. DADLE's inhibitory action on GP cells was significantly attenuated in 6-OHDA lesioned rats; 5% of GP cells studied (n=20) were inhibited >80% baseline, 40% of cells >20%, and 55% of cells were not significantly affected. Similarly, the sensitivity of GP cells to GABA was significantly decreased in lesioned rats. In naloxone treated lesioned rats, the reduced sensitivity of GP cells to DADLE was completely reversed while the cells remained subsensitive to GABA. These data show that 6-OHDA lesion causes a functional down regulation of opioid and GABA receptors in GP and that the former change is reversed by chronic naloxone treatment. The data suggest that dopamine denervation causes increased activity of striatopallidal enkephalin and GABA containing neurons.

461.12

OPIATE MODULATION OF DOPAMINE RELEASE IN THE STRIATUM AND NUCLEUS ACCUMBENS AS REVEALED WITH MICRODIALYSIS PROCEDURES. A. Mele*, P. Glue*, A. Pert (SPON: T. Bevan). BPB, NIMH and LCS, NIAAA.

Recent studies in rats, using microdialysis procedures, have demonstrated that systemically-administered mu opiate agonists enhance the release of dopamine in freely moving animals. The purpose of this study was to further localize this effect of opiates in brain using microdialysis techniques. Since opiate receptors appear to be localized on dopamine terminals in the caudate nucleus and nucleus accumbens, it was conceivable that the modulation of dopaminergic activity would take place at these levels. Unilateral concentric microdialysis probes were introduced into the caudate nucleus or nucleus accumbens of rats anesthetized with chloral hydrate. Both structures were dialyzed with artifical CSF or with various concentrations of morphine and opiate peptides. In our initial studies, racemic morphine sulfate was found to enhance the release of dopamine into the dialysate in a dose-dependent manner. DPDPE and DAGO produced similar effects. Unfortunately, however, these actions were only partially antagonized (25%) by naloxone. In addition, the (+) enantiomer of morphine was similar in potency with the (-) enantiomer. These findings suggest that opiate agonists probably do not exert their effects on dopaminergic function through dopamine terminals. Studies are underway to assess the participation of cell body regions.

461.13

THE PRESSOR RESPONSES OF CONSCIOUS RATS FOLLOWING INTRAVENOUS DYNORPHIN ADMINISTRATION ARE BLOCKED BY OPIOID, ALPHA ADRENERGIC AND VASOPRESSINERGIC RECEPTOR ANTAGONISTS. J.A. Thornhill, L.L. Gregor and Q.J. Pittman. Univ of Sask., Saskatoon, Sask. & Univ of Calgary, Calgary, Alta. Canada S7N 0W0.

Experiments were designed to determine the hemodynamic responses of conscious rats following intravenous (iv) dynorphin A_{1-13} administration and the receptor mechanisms mediating these changes. Male, Sprague-Dawley rats (≈300g body wt) were given iv bolus injections of dynorphin A_{1-13}, noradrenalin (NA), angiotensin II or sterile saline. IV administration of dynorphin A (20 nmoles/kg to 2 μmoles/kg) evoked dose-related pressor responses which were not attenuated upon repeated administration (i.e. no tachyphylaxis). Intravenous pretreatment with opioid antagonists (naloxone or MR2266) α-receptor antagonists, (yohimbine, prazosine or phentolamine)$_2$or the specific AVP-V_1 antagonist (d[$(CH_2)_5$-O-Me-(Tyr)^2, Arg^8] AVP given 10 min prior to iv dynorphin showed that all of these receptor antagonists could attenuate, or with higher concentrations, block the subsequent pressor response to iv dynorphin administration. These results suggest that in the conscious rat model the dynorphin A evokes a vasoconstrictor action, a response that can be blocked by kappa opioid as well as non-opioid (alpha adrenergic and vasopressinergic) receptor antagonists. This work was supported by the Canadian Heart Foundation.

461.14

KAPPA RECEPTOR MEDIATION OF VTA OPIOID-ELICITED FEEDING BEHAVIOR. Margaret E. Hamilton & Michael A. Bozarth. SUNY at Buffalo, Dept. of Psychology, Buffalo, NY. 14260.

The role of opioids in the ventral tegmental area (VTA) in feeding behavior was further explored. Opioid peptides were microinjected unilaterally in a volume of 0.5 μl into the VTA of non-deprived, freely moving rats and behavior was observed for 15 minutes. Dynorphin A (0--3.0 pmoles) or the Met-enkephalin analog DALA (0--3.0 nmoles), but not β-endorphin (0--100 pmoles) or the Leu-enkephalin analog DADLE (0--3.0 nmoles) elicited dose-dependent feeding. Similar to earlier findings between dynorphin(1-13) and morphine, dynorphin A was approximately 10,000 times more potent than DALA in producing feeding. This is consistent with the notion that the effect of opioids in the VTA on feeding may be mediated by kappa receptors. A role for mu receptors in the VTA in spontaneous feeding behavior remained possible, however, and kappa involvement required confirmation. The selective kappa agonist, U-50,488H (0--10 pmoles) elicited dose-dependent increases in total feeding durations. In contrast, total feeding was not significantly different from vehicle control among rats that received the mu agonist DAGO. These findings confirm that feeding elicited by opioids in the VTA is mediated by kappa receptors. It appears that kappa activation may promote the maintenance of naturally rewarding behaviors such as feeding.

461.15

ALTERATIONS IN POSTSYNAPTIC B-ADRENERGIC FUNCTION ACCOMPANY MORPHINE DEPENDENCE AND WITHDRAWAL. H.C. Moises AND C.B. Smith*+. Depts. of Physiology and Pharmacology+, University of Michigan, Ann Arbor, MI 48109.

We recently reported that B-adrenergic receptors are increased in various brain areas in rats after chronic morphine treatment and subsequently down-regulated during opiate withdrawal (Brain Res. 400, 1987). In this study, measurements of [3H]-dihydroalprenolol (3H-DHA) binding in hippocampus were carried out in conjunction with electrophysiological recording in vitro to determine whether such adjustments in receptor density might be of functional significance. Alterations in postsynaptic B-receptor function were assessed by comparing the ability of isoproterenol (ISO) to increase population spike responses and to block the calcium-dependent potassium after-hyperpolarization (AHP) in pyramidal neurons in hippocampal slices from saline and chronic morphine-treated rats.

Treatment of rats with increasing doses of morphine (up to 100mg/kg/inj., t.i.d.) for 14d resulted in a 19% in the Bmax for [3H]-DHA, whereas B-receptor density was decreased 27% in withdrawn subjects, compared to controls. No changes were found in the affinities of the receptors for [3H]-DHA or the agonists NE and ISO. Electrophysiological testing in CA1 revealed that extracellular responses to threshold (25nM) as well as maximal concentrations (500nM) of ISO were significantly enhanced in slices from dependent rats, whereas responsiveness to maximal concentrations of ISO was decreased, relative to controls, in slices from withdrawn subjects. In addition, the EC50 for ISO in reducing the AHP was significantly lower for dependent (360nM) and greater for withdrawn groups of slices (2.2uM), respectively, than for controls (820nM). These data suggest that long-term opiate administration and opiate withdrawal are accompanied by functional alterations in postsynaptic B-adrenergic systems (supported by NIDA grant DA-03365).

461.16

EFFECTS OF MONOAMINE ANTAGONISTS ON OPIOID-INHIBITION OF THE MILK-EJECTION REFLEX. G.Clarke & D.M.Wright. Dept. of Anatomy, Univ. of Bristol, & Upjohn Ltd. Crawley, U.K.

Opioids inhibit the milk-ejection reflex by suppressing the release of oxytocin, through more than one type of opioid receptor and at several sites. Monoamine and opioid systems are often interactive, therefore we examined the effect of monoamine antagonists on opioid inhibition of the milk-ejection reflex in the rat.

The opioids fentanyl (mu agonist) and U50-488H (kappa agonist) inhibited the suckling-evoked release of oxytocin. This inhibition was reversed by 1mg/kg and 10 mg/kg doses of naloxone respectively, and also by ketanserin (1mg/kg) a (S2 selective) serotonin antagonist. The β-adrenoceptor antagonist propranolol (1mg/kg) reduced the inhibition induced by U50-488H but not that of fentanyl. In contrast the other monoamine antagonists tested, phentolamine (α-adrenoceptor antagonist), haloperidol (dopamine antagonist) and cyproheptadine (non selective serotonin antagonist) were without effect.

The results suggest that: 1) Serotonin systems, acting through an S2 site, are involved in the inhibition of oxytocin secretion by both mu and kappa agonists. 2) The adrenergic system, acting through a α/β site, contributes to the kappa effect. (SPON: ENA)

461.17

MET-ENKEPHALIN STIMULATES ADRENERGICALLY-MEDIATED VASOCONSTRICTION IN RAT STRIATED MUSCLE.

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An endogenous opioid system may modulate peripheral vascular resistance in the rat. Small resistance vessels (6-15 μm) in rat striated muscle have demonstrated naloxone-sensitive constriction in response to topically administered methionine-enkephalin (MET). In this study, the mechanism of enkephalin-induced vasoconstriction was investigated using male, Sprague-Dawley rats, which were anesthetized with Na+ pentobarbital (50 mg/kg, i.p.). The microcirculation in the cremaster muscle (striated muscle) was observed using in vivo television microscopy. Arteriolar responses to topical application of MET (10^{-6}M) were observed before and after 1) the topical addition of phentolamine (PHE; 10^{-6}M), an alpha-adrenergic antagonist, 2) the topical addition of prazosin (PZN; 10^{-6}M), an alpha-1 adrenergic antagonist, and 3) local denervation with 6-hydroxydopamine (6HD; 10^{-6}M). MET induced vasoconstriction (66 ± 7 % of control diameter), which was abolished in the presence of PHE (101 ± 1%), PZN (108 ± 4%), and 6HD (108 ± 7%). These data suggest that met-enkephalin functions by stimulating the release of norepinephrine from sympathetic nerves supplying the striated muscle microcirculation. (Supported by grants from the Amer. Heart Assoc. and the Univ. of Louisville Med. Sch.).

461.18

OPIOID AGONISTS AND THE REGULATION OF NEUROTUMOR GROWTH.

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Neural tumor tissue is known to possess endogenous opioids and opioid receptors. A study was conducted to determine which opioid ligand-receptor complex is most effective in modulating neuro-oncogenesis. Ajax mice were inoculated with 10^6 S20Y neuroblastoma cells and injected daily with methionine enkephalin (ME)(0.5-30 mg/kg); tumor size and appearance, and survival were monitored. On day 12, a time when 100% of control mice had a tumor, the number of mice having measurable tumors (>5 mm) was significantly reduced for mice receiving 0.5-30 mg/kg ME. At the time when 100% of control mice were dead, 10-33% of mice in the ME groups were alive. The antitumor effects of ME on tumor growth were reversed by naloxone. Study of other opioid agonists showed that daily injection (10 mg/kg) of DADLE or EKC, prototypic ligands for δ and κ receptors, respectively, had no effect on tumorigenic events. These results corroborate tissue culture studies identifying ME as a potent and natural trophic agent influencing neural cancer. Supported by NIH grant NS-20623.

461.19

PAIN PERCEPTION IN THE RAT IS MODULATED BY GONADAL STEROIDS.

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In the rat, gonadal steroids influence central and peripheral levels of the endogenous opioid peptide, beta-endorphin. As an assessment of total endogenous opioid activity, the time to respond to a noxious somatic stimulus (tail flick latency and paw lick/jump lactency) was measured in castrated and castrated gonadal steroid replaced male and female rats. Rats were castrated for two weeks prior to testing. At the end of the third week, gonadal steroid treatment was initiated and maintained for three weeks in half of the animals, while the remaining animals received the vehicle and served as castrated controls. The tail flick latency was significantly increased in castrated male rats at 1, 2 and 3 weeks of testosterone proprionate (TP) treatment. The time to paw lick or jump was greater in castrated male rats only after 3 weeks of treatment with TP. In castrated female rats the tail flick latency was increased 2 and 3 weeks after estradiol benzoate (EB) treatment was begun, while the time to paw lick or jump was reduced with 2 and 3 weeks of treatment with EB. These data indicate that gonadal steroids modulate the perception of pain in male and female rats and that the this modulation may vary according to sex and the stimulus employed.

461.20

MODIFICATION OF PERIPARTURITIONAL PAIN THRESHOLD BY INGESTION OF AMNIOTIC FLUID IN THE RAT.

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Amniotic fluid (AF) and placenta contain a substance(s) (placental opioid-enhancing factor, POEF) that when ingested by nonpregnant rats, enhances (but does not cause) any opiate-mediated analgesia they are experiencing (e.g., morphine, or vaginal/cervical stimulation): 0.25 ml amniotic fluid or 1 placenta being the optimal doses for enhancement of a 3 mg/kg morphine injection.

Preliminary data suggested that opioid enhancement is a significant benefit of amniotic-fluid- (and placenta-) ingestion to the mother during the periparturitional period. Depriving parturient rats of amniotic fluid, is apparently impossible to do without interfering with delivery, stressing the mother inordinately, or interfering with the measurement of pain threshold, we have been trying to get at the answer indirectly by infusing amniotic fluid in the last few hours before the start of delivery (before the mother normally has access to it), and examining changes in enhancement of pregnancy-induced analgesia, as measured by tail-flick latency in a hot-water tail-dip test.

We assessed the effect of 3 doses of AF on pain thresholds in late-pregnant rats both with and without naloxone pre-treatment. They were assessed prepartum, after the delivery of the 1st pup, and 40 min and 2 hrs after delivery of the last.

Results: (a) larger doses produce greater enhancement; (b) the longer the Interval between the Prepartum test and the Onset of Delivery (the IPrOD), the greater the enhancement. Prepartum AF lowers pain thresholds during parturition and after delivery, if the dose of AF is low and the IPrOD is short. Naloxone pretreatment lowers pain thresholds during (a) the prepartum, parturitional, and postpartum period in AF-fed rats, (b) the prepartum period in control rats, and (c) the parturitional and postpartum period in control rats with short IPrODs.

Mother rats do show increased analgesia resulting from ingestion of amniotic fluid in the periparturitional period. The enhancement is complex, however; POEF may have a bi-phasic action, and may even enhance opioid antagonists such as naloxone.

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION IV

462.1

EFFECTS OF NAALADASE INHIBITORS ON [^{3}H]NAAG METABOLISM *IN VIVO*.

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The endogenous brain peptide, N-acetyl-aspartyl glutamate (NAAG), has been proposed as an excitatory neurotransmitter. Recently, a quisqualate (Quis) and phosphate-sensitive membrane bound metallopeptidase, NAALADase, which cleaves NAAG to glutamate and N-acetylaspartate, has been identified and purified. To determine whether NAALADase plays a role in the catabolism of NAAG *in vivo*, we have examined the effects of intrastriatal co-injection of NAALADase inhibitors on [^{3}H]NAAG metabolism. [^{3}H]NAAG (10 pmoles), radiolabelled on the glutamate residue, was dissolved in isotonic saline and infused by a microliter pump via a cannula stereotaxically placed in the striata of rats anesthetized with pentobarbital. The rats were decapitated 5 min after the infusion and their striata were quickly dissected on ice and extracted in ice-cold methanol. [^{3}H]NAAG and [^{3}H]glutamate were separated by ion exchange chromatography over AG1-X8 resin. The apparent $t_{1/2}$ for [^{3}H]NAAG degradation in striatum was 3.4 min. Co-injection of 25 mM phosphate (IC_{50} = 100 μM) slowed the $t_{1/2}$ of [^{3}H]NAAG to 7.8 min; the addition of 100 pmoles of Quis (IC_{50} = 0.5 μM) further inhibited metabolism by an additional 35%. To control for the potential excitatory effects of Quis, 100 pmoles of kainic acid (IC_{50} > 100 μM) and of NMDA (IC_{50} > 100 μM) were co-injected but were devoid of inhibitory effects on [^{3}H]NAAG metabolism. Co-injection of dithiothreitol (50 mM), which presumably binds the metallo-cofactor of NAALADase, inhibited [^{3}H]NAAG metabolism by 38%. Thus, co-injection of agents that inhibit NAALADase activity in three different ways prolongs NAAG catabolism *in vivo*, consistent with a role in endogenous NAAG disposition.

462.2

PURIFICATION OF N-ACETYLATED ALPHA-LINKED ACIDIC DIPEPTIDASE (NAALADASE) FROM RAT BRAIN AND KIDNEY.

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Based on electrophysiological, immunocytochemical and release studies, N-acetyl-aspartylglutamate (NAAG) is a putative excitatory neurotransmitter in the mammalian CNS. One potential mechanism for NAAG inactivation is via a dipeptidase (NAALADase), which degrades NAAG to N-acetylaspartate (NAA) and glutamate and may be coupled to sodium-dependent high affinity glutamate uptake. This peptidase is restricted to kidney and neural tissue.

We have solubilized the peptidase from both brain and kidney with Triton X-100 followed by sequential column chromatography. Both brain and kidney NAALADase are resolved into two peaks over DEAE-Sepharose. The activity of all peaks is inhibited by preincubation with EGTA or dithiothreitol, by quisqualate and phosphate, and is activated by cobalt; the inhibition or activation occurs at similar concentrations to that observed for the native membrane-bound peptidase. The first DEAE peak of brain NAALADase activity was further purified with CM- and Concanavalin A-Sepharose and Sepharose CL-6B chromatography; on each of these columns only one peak of activity was observed. We estimate that NAALADase is enriched over 5,000-fold by this purification; however, the activity is unstable after the DEAE step. Size exclusion chromatography suggests a molecular weight of approximately 190 kDa. Silver stained SDS gels of fractions surrounding the NAALADase peak of activity from CM- and size columns show enrichment of a 90 kDA band. Only 5 protein bands remain after these steps. NAALADase, defined as a quisqualate- and phosphate-sensitive metallopeptidase which degrades NAAG to NAA and Glu, is the only route of NAAG degradation observed to date.

462.3

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO N-ACETYL-ASPARTYL-GLUTAMATE.

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N-acetyl-L-aspartyl glutamate (NAAG) is thought to be a putative neuromodulator/neurotransmitter in the mammalian nervous system. Immunohistochemical studies with polyclonal NAAG antisera have revealed immunoreactive neurons and processes in several brain regions. However, these antisera crossreacted to some degree with N-acetyl-aspartate (NAA), which is present in mM concentrations in brain, prompting the development of monoclonal antibodies more specific for NAAG.

By fusing spleen lymphocytes from a BALB/c mouse preimmunized with NAAG-bovine serum albumin conjugate with SP2/0-Ag 14 mouse myeloma cells, we produced three monoclonal IgG 2a (kappa) antibodies that specifically reacted with NAAG. These antibodies did not crossreact with NAA nor with structurally similar peptides as shown by solid phase radioimmunoassay. Antibody activity was absorbed out selectively by both NAAG-thyroglobulin conjugate and free NAAG. These monoclonal antibodies stained numerous nuclei of the medulla-pons and midbrain, mitral cells in the olfactory bulb, pyramidal neurons in sensorimotor cortex, locus coeruleus and several cholinergic cranial nuclei. The staining pattern strongly correlated with NAAG levels determined by HPLC. Monoclonal antibodies significantly enhanced sensitivity of staining, allowing visualization of dorsal horn neurons in spinal cord which were not readily detectable with polyclonal antiserum.

Availability of these monoclonal antibodies now facilitates further clarification of the role of NAAG in the brain.

462.4

CO-VARIANCE OF NAAG LEVELS AND NAALADASE ACTIVITY IN ADULT RAT CNS: A MICROPUNCH STUDY.

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NAAG is an acidic neuropeptide which has been implicated in excitatory neurotransmission. NAALADase is a membrane bound, quisqualate sensitive metallopeptidase specific for N-acetylated, α-linked acidic dipeptides which cleaves NAAG to N-acetyl-aspartate and glutamate. Both NAALADase and NAAG are nonuniformly distributed across rat CNS, although the correlation between their localizations is unclear. In order to elucidate the *in vivo* relationship between NAAG and NAALADase, we examined the levels of both in micropunch dissections in rat CNS. Furthermore, we examined the response of NAALADase activity to enucleation of the eye.

Micropunch dissections of 21 discrete brain nuclei and spinal cord regions were assayed for both NAALADase activity and NAAG levels. NAALADase activity was determined by the amount of [^{3}H]NAAG which was metabolized to [^{3}H]glutamate. NAAG was quantitated using a radioimmunoassay. NAALADase activity exhibited a 5-fold variation among the structures assayed. The correspondence between peptidase activity and NAAG levels was variable. Regions where both the peptidase and the neuropeptide were in relatively high amounts include locus coeruleus and dorsal raphe. A region where NAALADase activity was high while NAAG levels were low is cerebellum. Low NAALADase activity and high NAAG levels occurred in the optic tract and red nucleus. To investigate the regulation of the peptidase in response to altered levels of NAAG, rats were enucleated unilaterally with a subsequent determination of NAALADase and NAAG in the optic tract and superior colliculus at 1, 2, 3, and 4 weeks after the lesion. While NAAG levels decreased in the optic nerve ipsilateral to the lesion and in the contralateral superior colliculus, NAALADase activity increased in both regions. These results suggest that NAALADase may have another role *in vivo* in addition to the degradation of NAAG. The up-regulation of the peptidase activity in response to neuronal degeneration indicates that the peptidase may be expressed in non-neuronal cells.

462.5

CALCIUM-DEPENDENT EVOKED RELEASE OF [^{3}H]N-ACETYLASPARTYLGLUTAMATE FROM THE OPTIC TRACT.

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N-acetylaspartylglutamate (NAAG) is a neuropeptide localized to several glutamatergic systems, including the rodent optic pathway. To determine whether the peptide is released by depolarization, the superior colliculus of the rat was perfused with 2 μCi of [^{3}H]NAAG followed by one hour with Krebs bicarbonate buffer, using a microdialysis system. Subsequently, 10 min fractions were collected and analyzed for [^{3}H]NAAG by HPLC. Addition of 100 μM veratridine resulted in a eight-fold increase in the evoked release of [^{3}H]NAAG, which was virtually abolished by co-perfusion with Krebs buffer devoid of Ca^{2+}, containing 1mM EGTA. The basal level after labeling with [^{3}H]NAAG appears to be associated predominantly with glutamate, and veratridine depolarization has little effect on this release. Prior enucleation of the right eye reduced the basal level of [^{3}H]NAAG release by 50%, and the veratridine evoked release by greater than 85%, from the left superior colliculus. Preliminary studies indicate that [^{3}H]glutamate subjected to the same procedure of labeling, washout and evoked release results in less than two-fold increase in release of tritium associated with NAAG on HPLC. Thus, with [^{3}H]NAAG as the substrate, a more specific labeling of a discrete population of presumed nerve terminals may be accomplished than when [^{3}H]glutamate itself is used as a precursor. These results suggest that NAAG is released upon depolarization and may serve as a neurotransmitter/neuromodulator in the optic tract.

462.6

IDENTIFICATION AND PURIFICATION OF RAT BRAIN T-KININOGEN.

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T-kinin (Ile-Ser-bradykinin) present in tissues and body fluids is derived from trypsin sensitive T-kininogens, one of which is identical to plasma α$_1$-major acute protein (α$_1$-MAP). The latter increases in adjuvant treated rats or ones with experimental inflammation. In studies on cysteine proteinase inhibitors (CPI's) from rat brain, we isolated from cytosol and membrane fractions an 80 kDa glycoprotein liberating kinin(s) upon incubation with trypsin but not glandular kallikrein when assayed on the isolated rat uterus. Inhibitor was purified by alkylated papain affinity chromatography and shown by immunoblots to cross-react with a histidine-rich glycoprotein (HRG) but not anticystatin C, antilysozyme or anti-β-protein amyloid. Rat brain contained 0.5 nmol per g wet weight. The 80 kDa CPI suppressed at low concentration brain cathepsin L cleavage of Leu-enkephalin at Gly-Gly bond (to yield Tyr-Gly) with K_i 10^{-11} M. T-kininogen was present in all subcellular fractions including nuclear (P1) and microsomal (P3) particulates, post-microsomal supernatant (S3), but was the only CPI found in P2-synaptosomes. The presence of CPI's in high concentration is of interest to lysosomal regulation, protein turnover, formation of neuropeptides (T-kinins) or abnormal (amyloid) deposits.

Supported in part by grant NIDA 04178.

462.7

HUMAN DBI AND ITS PROCESSING PRODUCTS IN BRAIN AND CSF.

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The structure of an 11k Da polypeptide, termed DBI (Diazepam Binding Inhibitor) purified from human brain (H-DBI) was determined by recombinant DNA technology. In rat brain DBI is processed into 3 biologically active allosteric modulators of $GABA_A$ receptor subtypes. We wanted to test whether H-DBI, which similar to rat DBI elicits a proconflict effect and inhibits beta-CCM binding, is processed in human brain into natural allosteric modulators of $GABA_A$ receptor subtypes. The technology for the purification of these H-DBI putative processing products, using specific antibodies raised in rabbits against fragment 51-70 (eikosaneuropeptide, ENP), will be reported. ENP was first obtained through tryptic digestion of purified H-DBI and shown to be as potent as H-DBI in eliciting proconflict behavior in rats. In acetic acid extracts of human brain tissues or CSF at least two peaks of ENP-like immunoreactivity were detected. While we are obtaining the amino acid sequence of these putative natural ligands, we are studying the content of DBI and its processing product in the CSF of patients suffering from various neuropsychiatric disorders.

462.8

TYROSYLPROTEIN SULFOTRANSFERASE: DISTRIBUTION AND POST-NATAL DEVELOPMENT.

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Sulfation of tyrosine residues is a ubiquitous post-translational modification of biologically active peptides and proteins which is catalyzed by the golgi enzyme, TPST. The activity was measured using a filter paper assay with PAPS and Poly Glu_6,Ala_3,Tyr_1 as the sulfate donor and acceptor substrate, respectively. TPST activity was found in crude microsomal fractions prepared from livers of rabbit, rat, mouse, hamster and human. In rat, TPST activity was highest in liver, although appreciable activity was also seen in lung, spleen, heart, kidney and salivary gland. In rat brain TPST was found to be most abundant in the pituitary and cerebellum although, significant activity was also found in the hypothalamus, striatum, cerebral cortex and hippocampus. TPST activity in liver increased during post-natal development approximately five-fold from day 1 after parturition to day 5, at which time adult levels were attained. In contrast, rat cerebellar TPST activity increased only 2.5-fold from Day 1 to Day 8 at which time activity began to decline, reaching adult levels around Day 28. In neither the rat liver nor cerebellum were any significant differences in TPST activity observed during development of male or female rat pups. (Supported by NIH Grant ES20530 and by a PMAF Fellowship)

462.9

ROLE OF AN α -HELIX IN THE RECOGNITION OF DIAZEPAM BINDING INHIBITOR (DBI) FOR THE DIFFERENT TYPES OF BENZODIAZEPINE (BZ) RECOGNITION SITES.

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To interpret in structural terms interactions of rat DBI with BZ recognition sites, we used secondary structure prediction methodology (Chou, P.Y., Fasman G.D., Biochemistry-13; 222, 1974) and analysis of hydrophobicity (Kyte, J., Doolittle, R.F., J. Mol. Biol. 157; 105; 1982). We synthetized the DBI peptides(17-50),(18-50),(22-50),(26-50) and (33-50) by solid phase methodology. CD spectrum of DBI (17-50), (18-50), (22-50) demonstrated α -helical structure in MeOH. Pharmacological studies revealed that these peptides injected intraventricularly in rats causes proconflict activity. In primary cultures of cerebellar granular cells DBI peptide (33-50), displaced [^{3}H]flumazenil with K_i of $\sim$ 5 μM; the other synthetic peptides were ineffective in concentrations up to 200 μM. In primary culture of the cerebellar glial cells the peptides with stable α -helical structure DBI (17-50), (18-50), (22-50) were able to displace [^{3}H]PK11195 with K_i $\sim$ 10 uM. DBI (26-50)and (33-50) which lack the α-helical part of their structure were ineffective.

462.10

PURIFICATION FROM BRAIN OF AN ORGANIC INHIBITOR OF ^{3}H-OUABAIN BINDING.

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Whole rat brains were dried and defatted using separate acetone and petroleum ether extractions. After 0.2 M acetic acid extraction the peptide fraction was chromatographed on a Sephadex G-25 column and the fractions were monitored using the Lowry reaction. Those fractions of 1,000 to 5,000 daltons were rechromatographed on a Bio Rad AG-50 column to remove sodium and potassium. 100 ug of either fraction 4 or 5 reduced ouabain binding to 83% of control. These fractions did not inhibit naloxone, spiperone, verapamil or muscarinic binding. This material (5 mg) was separated using HPLC on a C_{18} uBondapak column. A linear gradient of 0.1% trifluoroacetic acid (TFA) in water to 100% 0.1% TFA in acetonitrile resolved two peaks of ouabain inhibiting activity. The smaller more hydrophobic peak produced a 10% decrease in ouabain binding. The hydrophilic peak was rechromatographed using a slower gradient (only to 50%-0.1% TFA in acetonitrile. This material weighed 1.5 mg, was 18% protein and 100 ug decreased ouabain binding to 78% of control. Ashing (600°C for 1 hr) completely abolished activity. (Supported by the Health Sciences Centre Research Foundation, the Medical Research Council of Canada and the University of Manitoba).

462.11

PURIFICATION OF L-ASPARTATE N-ACETYLTRANSFERASE FROM RAT BRAIN,

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L-aspartate N-acetyltransferase (ANAT), a nervous system specific enzyme, mediates the acetylation of L-aspartate by acetyl-coenzyme A. The product, N-acetylaspartate, is a predominant acidic amino acid in the nervous system and may serve as a precursor in the biosynthesis of the dipeptide, N-acetylaspartylglutamate.

ANAT was solubilized from brain membranes in 1% Triton X-100 in 10 mM phosphate buffer, pH 6.8. The solubilized enzyme passed through a DEAE-anion exchange column at pH 7.0 and the unbound fraction, containing most of the enzyme, was further purified by anion exchange HPLC at pH 8.1. The active peak fractions were pooled and subjected to gel filtration HPLC followed by affinity chromatography on an L-aspartate immobilized column. The eluate showed a 600-fold increase in specific activity and 2-3% yield.

This purification will support analyses of the regulation and cellular distribution of ANAT, contributing to an understanding of the function of N-acetylaspartate. (grant DA 02297)

462.12

NEWLY SYNTHESIZED PEPTIDE IS FIRST TRANSPORTED TO RELEASE SITES: AUTORADIOGRAPHIC EVIDENCE FROM A CRAB NEUROSECRETORY SYSTEM.

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Preferential release of newly synthesized peptide has been found for all neurosecretory systems tested. This study shows that granules containing biosynthetically radiolabelled material are first transported to release sites. Later they become intermixed with non-labelled granules. The X-organ - sinus gland system of crabs (Cardisoma carnifex) was isolated and arranged to permit independent perfusion of the somata (X-organ) and terminals (sinus gland). The somata were given a pulse (5 min to 5 h) of ^{3}H-leucine in crab saline with glucose, while the terminals were continuously perfused with nutrient medium. Systems were chased for 1 - 72 h. The material was examined by light- and EM autoradiography (7 d exposures). At 1 h, exposed grains occur primarily over granules in Golgi. At 10 h, radiolabel is in the axon tract with little in the terminals. By 19 h, label is found preferentially in terminals abutting the internal hemolymph sinuses. After 72 h, label is evenly distributed throughout release and storage sites. Exocytotic profiles, indicative of release sites, have only been observed where terminals abut a hemolymph sinus (Weatherby, 1981, Cell Tissue Res. 220:293). Transported label represents hormonal peptides, and these are secreted by a Ca-dependent mechanism (Stuenkel, 1983, J. Comp. Physiol. **B153**:191; 1985, J. Physiol. **359**:163). Thus, the autoradiography provides a physical basis for preferential release of newly synthesized peptides. Supported by NSF BNS84-04459 and NIH NS 15453.

462.13

DETERMINATION OF NEUROPEPTIDES AND CYCLIC-AMP BY HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS.

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Radioimmunoassays for neuropeptides and cyclic-AMP are time consuming because they are based on radiolabeled compounds with segregation of a mixture performed by antibodies. Liquid chromatography can resolve a mixture of neuropeptides, but is not sensitive enough for some applications. We overcame some of these difficulties using high performance capillary electrophoresis (HPCE). Separation of a mixture of peptides and cyclic-AMP was performed in an untreated fused silica microbore tube (75 um x 100 cm) using a mobile phase of 0.05 M sodium tetraborate, pH 8.3. The test mixture contained neurotensin, met-enkephalin, leu-enkephalin, substance P, angiotensin II, sulfated cholecystokinin-8 and cyclic-AMP. Detection was performed with a modified Hitachi UV detector. Samples (4 nl) were electrokinetically loaded applying 10 KV for 15 sec, and electroosmotic flow for separation was created with 22 KV. By reversing polarity the peptide zones were run forward and backward to determine the UV-spectrum of each substance. Linearity was tested with eight different concentrations of the mixture (range 10^{-4} M to 10^{-6} M). For all compounds the regression coefficient relating concentration of the standard to absorbance units was equal to 1.00. Compounds were well separated and the retention time error was less than 2% in nine different runs. Sensitivity was between 0.1 and 0.01 picomoles, and quantification of a sample lasted about 20 min per run. These results show that HPCE is reliable for neuropeptide and cyclic-AMP analysis.

462.14

GLIAL ASSOCIATION OF ANGIOTENSINOGEN (A_0) mRNA DEMONSTRATED BY COMBINED IN SITU HYBRIDIZATION AND GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IMMUNOCYTOCHEMISTRY.

R.L. Stornetta, P.G. Guyenet, C. Hawelu-Johnson* and K.R. Lynch*. Dept. of Pharmacol., Univ. of Virginia School of Med., Charlottesville, VA 22908

Distribution of rat brain A_0 mRNA and in particular, the cell types associated with A_0 mRNA in brain, was determined by using in situ hybridization combined with immunocytochemistry in single brain sections. Two different hybridization probes for A_0 mRNA were used: a ^{35}S-labelled 42 residue oligonucleotide and a full-length A_0 RNA (antisense or sense) labeled with tritiated UTP. Rats were anesthetized with pentobarbital (60 mg/kg, ip) and perfused transcardially with 4% paraformaldehyde. Brains were extracted, cryoprotected and sections cut 10 μm thick on a cryostat and placed into prehybridization solution for 4-18 hrs at 37° C. Either ^{35}S-labelled oligonucleotide or ^{3}H-labelled sense or anti-sense A_0 mRNA was added to the sections at a concentration of 2-3 nM. Thirty-50 fold excess unlabelled 42-mer oligonucleotide was added to some sections as a control in the ^{35}S experiments. Sections were incubated at 37° C for 18 hrs (^{35}S probe) or 72 hrs (^{3}H probe), then washed though a graded series of salt solutions of decreasing concentrations with a final wash series with RNAase (for ^{3}H- labelled RNA) at 37° C followed by a 42° C wash (both probes). Sections were postfixed in 4% paraformaldehyde and standard peroxidase-anti-peroxidase immunocytochenistry for GFAP or microtubule-associated protein-2 (MAP, a neuronal marker) was performed. Sections were then mounted onto subbed slides, dehydrated, dipped in NTB2 emulsion and exposed at 4° C for 10-14 days (^{35}S) or 4-6 weeks (^{3}H) and developed with D-19. Clusters of silver grains were seen throughout the brain with higher concentrations in ION, NTS, PBN and many hypothalamic nuclei. Grains were primarily clustered over GFAP reactive cell soma and processes. Most grain clusters were not associated with MAP reactive cells. This confirms our previous findings that the distribution of brain A_0 mRNA overlaps many of the areas reported to contain high levels of angiotensinogen and further suggests that angiotensinogen in brain is synthesized primarily by glia. HL33513

462.15

ALPHA BAG CELL PEPTIDE CAUSES PARALLEL REDUCTION OF STIMULATED cAMP LEVELS AND PRO-ELH BIOSYNTHESIS IN APLYSIA BAG CELLS. R.W. Berry. Dept. Cell Biology and Anatomy, Northwestern Univ. Sch. of Med., Chicago, IL 60611.

Alpha bag cell peptide (α-BCP), one of the multiple secretory products of the precursor to the egg-laying hormone (proELH) of bag cells, has been reported to exert excitatory (Rothman, et al., PNAS, 80:5753, 1983) or inhibitory (Kauer, et al., J. Neurosci., 7:3623, 1987) feedback on the cells which secrete it. Since pro-ELH synthesis in these cells is modulated by cAMP, it was of interest to determine if α-BCP causes parallel alterations in cAMP levels and precursor synthesis. In this study, the peptide had no effect on unstimulated bag cell cAMP levels or on elevation of cAMP levels by IBMX, indicating a lack of effect on basal adenylate cyclase activity. However, it reduced the cAMP elevations induced by forskolin, high potassium, and dopamine. Consistent with the hypothesis that proELH synthesis is accelerated by cAMP, α-BCP reduced precursor synthesis and cAMP production in parallel in forskolin-stimulated preparations, but did not reverse the stimulation of peptide synthesis caused by IBMX or depletion of intracellular Ca^{++}.

MESSENGER RNA REGULATION IV

463.1

C-FOS mRNA EXPRESSION IN BRAIN INDUCED BY SUBCONVULSIVE DOSES OF CAFFEINE. T. Nakajima, J.L. Daval, P.F. Morgan, and P.J. Marangos[1]. Unit on Neurochemistry, BPB, NIMH, Bethesda, MD 20892 and [1]Gensia Pharmaceuticals, San Diego, CA 92121-1207.

In several previous reports, the proto-oncogene c-fos was shown to be induced by occupation of nicotinic acetylcholine receptors and depolorization-induced calcium influx in PC12 cells. It is also induced following various seizures. Caffeine is a non-selective adenosine receptor antagonist as well as a convulsant at high doses. We examined caffeine-induced c-fos expression and its pharmacological interaction at the mRNA level in mouse brain. Caffeine administration (17.5mg, 32mg, 56mg, and 100mg/kg i.p.) increased dose-dependently c-fos mRNA levels and at the convulsive dose (175mg/kg i.p. ED50 133mg/kg) c-fos levels were highest. A time-course study revealed that after caffeine injection, c-fos levels increased rapidly to reach a maximum at 15-30 minutes and declined progressively to basal levels at 4 hours. The pharmacological profile of subconvulsive caffeine-induced c-fos expression was also investigated, and suggests that both benzodiazepines and adenosinergic mechanisms may be involved in this system.

463.2

TRANSIENT CEREBRAL ISCHEMIA (TCI) INDUCES C-FOS ONCOGENE EXPRESSION IN THE RAT BRAIN. D.R. Gehlert, M.B. Jørgensen*, J Deckert and D.C. Wright. ETB and SNB, NINCDS, N.I.H., Bethesda, Maryland 20892, Neuropatologisk Institut, Copenhagen, Denmark and Universitats-Nervenklinik, Fuchsleinstr., Wurzburg, FRG.

The c-fos protein is a cellular product that appears in neurons rapidly following neuronal stimulation. The c-fos proteins are found in the nucleus and appear to be associated with chromatin. The c-fos protein has been implicated in changes in the long term function in cells such as those which may occur in learning and memory. The basal levels of c-fos in the brain are very low but are reported to increase rapidly following sensory stimuli. Therefore, we have examined c-fos mRNA in the brain following TCI.

Under anesthesia, the vertebral arteries of male, Sprague-Dawley rats were cauterized and the common carotid arteries were isolated and exposed. Twenty-four hours later severe forebrain ischemia was induced by occluding the common carotid arteries. Animals were killed at various time periods thereafter from 3 to 72 hours. The brains were removed and sectioned at 20 microns. Following formaldehyde post fixation, the sections were hybridized using a riboprobe labelled with ^{35}S-nucleotides (Lofstrand, Gaithersburg, MD). Hybridization was detected by autoradiography using sheet film or Kodak NTB-2 emulsion.

TCI resulted in a dramatic increase in hybridization for c-fos mRNA in the brain. In general, the expression followed the distribution and time course of TCI-induced damage in the brain. High levels of expression were seen in individual neurons of the caudate-putamen, CA1 of the hippocampus and in scattered neurons in the hilus of the area dentata. These results indicate that c-fos may play a role in the damage and recovery of brain neurons following TCI.

463.3

ADRENERGIC DRUGS PROMOTE CHANGES IN C-FOS mRNA LEVELS IN BRAIN. R.M.Gubits,T.Smith*+,J.L.Fairhurst* & H.Yu* Dvsn.of Pediatric Neurology,Columbia Univ. Coll. of Phys. & Surg.,NYC, NY 10032,+Dvsn.of Analyt. Pharm.,Nathan Kline Institute, Orangeburg, NY, 10962.

The proto-oncogene c-fos encodes a nuclear phosphoprotein, which is thought to act as a transcriptional factor for other specific genes. c-fos gene expression is readily detectable in adult mammalian brain and transiently inducible in neurons by seizure activity, cholinergic receptor agonists and depolarization. To examine the in vivo effects of adrenergic drugs on c-fos mRNA expression in brain, adult male rats were injected ip as follows:1.)vehicle (V) alone,2.)yohimbine (Y) (alpha2 antagonist) alone,3.)clonidine (C) (alpha2 agonist), propranolol (P)(ß-antagonist),or prazosin (Pz) (alpha1 antagonist), alone or preceding yohimbine injection. We observed a transient induction of c-fos mRNA after vehicle injection, which was enhanced and prolonged by Y injection. Either C or P suppressed the vehicle induction,while C,P, and Pz suppressed induction of c-fos mRNA by Y. These data are consistent with the hypothesis that c-fos mRNA levels increase as a result of the interaction of norepinephrine (NE) with the post-synaptic ß-adrenergic receptor. According to this interpretation, the effects of Y and C would be due to modulation of NE release by the autoinhibitory effect of the pre-synaptic alpha2 adrenergic receptor. Supported by NIH/GM38202 and Colleen Giblin Foundation.

463.4

LOCALIZATION OF INCREASED *c-fos* mRNA CONTENT IN RAT CNS FOLLOWING RECURRENT SEIZURES. Christine Gall, Amy Arai*, and Jeffrey White, Dept. of Anatomy and Neurobiology, Univ. of California., Irvine, CA 92717 and Div. of Endocrinology, SUNY, Stony Brook, NY 11794.

The product of the cellular proto-oncogene *c-fos* has been suggested to be a regulatory factor in gene transcription. Recently, several groups, including ourselves, have demonstrated that *c-fos* protein and/or mRNA content in the CNS is increased in response to seizures. In the present study, *in situ* hybridization autoradiography was used to evaluate the correspondence between changes in the abundance of mRNAs for *c-fos* and preproenkephalin A following seizure activity.

Bilateral recurrent seizures were induced by either unilateral electrolytic lesion of the dentate gyrus hilus (HL) or perforant path stimulation in anesthetized male rats. Perfusion-fixed tissue sections through forebrain of experimental and paired control rats were processed for the localization of *c-fos* mRNA using a ^{35}S-cRNA probe. Within 15min. of electrically stimulated seizure initiation, hybridization was increased exclusively within the dentate gyrus granule cell layer, bilaterally. At 3hrs post-HL, hybridization to *c-fos* mRNA was reliably greatly elevated within dentate gyrus stratum granulosum and layer II of piriform and entorhinal cortices. Modest increases in hybridization were also generally evident throughout hippocampal stratum pyramidale, olfactory tubercle, and anterior olfactory nucleus. By 6 hrs post-HL, hybridization density had declined somewhat in stratum granulosum whereas labeling of entorhinal cortex had not diminished from the 3hr time point. These data indicate an extremely rapid increase in the abundance of *c-fos* mRNA in most, but not all, areas in which preproenkephalin A mRNA becomes elevated in the same paradigm. The data are consistent with *c-fos* gene activation in response to seizures but further work, including examination of additional time points, is needed to determine if *c-fos* transcription is elevated in all areas which exhibit seizure-induced increases in enkephalin synthesis.

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463.5

REGIONAL DISTRIBUTION OF EPIDERMAL GROWTH FACTOR mRNA IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM. L. M. Lazar*, J. L. Roberts and M. Blum. (SPON: David E. Wolfe) Fishberg Center for Neurobiology, Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Epidermal growth factor (EGF) is a mitogenic polypeptide, originally isolated from the male mouse submaxillary gland, which has been found to exert a neurotrophic effect on primary neuronal cultures. Recent investigations into the localization of EGF within the mammalian central nervous system have identified regions of mouse and rat brain which contain relatively high levels of EGF-immunoreactive material. While such demonstrations of EGF in the CNS might reflect protein sequestration and not brain-specific protein synthesis, another study utilizing dot-blot analysis has detected preproEGF mRNA in whole mouse brain preparations suggesting local CNS synthesis. Our studies use a more sensitive RNA quantitation assay to focus on the regional distribution of EGF-specific mRNA within the central nervous system of the adult male mouse in order to further explore the brain-specific expression and potential neurotransmitter-neuromodulator functions of this neuropeptide. Using a solution hybridization S1 nuclease protection assay, we have been able to detect as well as quantitate the levels of EGF-specific mRNA in several brain regions of the adult male mouse. Our preliminary studies reveal EGF-specific mRNA at levels of approximately 50 fg per ug total RNA in mouse cerebellum and striatum with no detectable EGF mRNA in cortex. We are currently investigating the developmental pattern of expression in these tissues.

463.6

IN SITU HYBRIDIZATION ANALYSES OF CA++/CALMODULIN-DEPENDENT PROTEIN KINASE II IN DEVELOPING RAT BRAIN. K. Burgin*, M. N. Waxham* and P. Kelly (SPON: R. Yip). Dept. of Neurobiol. and Anat., and Neurology, Univ. of Tex. Med. Sch., Houston, TX 77225.

Ca++/calmodulin-dependent protein kinase II (CK-II) is a multimeric enzyme consisting of nonstoichiometric amounts of two subunits, alpha (50K) and beta (60K). Although this kinase is highly concentrated in brain tissue, immunocytochemical analyses have shown that its distribution among different brain regions is highly variable. We have recently shown that the two subunits are expressed at different times and rates during rat forebrain development. In the present study we have extended earlier findings to the mRNA level. Using oligonucleotide probes specific for each subunit we have examined changes in subunit-specific mRNA levels and their distribution during rat brain development.

While the level of alpha subunit protein is barely detectable at postnatal day 5, its mRNA is already prominent at 4 days and exhibits a distribution similar to that described for the protein in adult brain. The strongest hybridization was associated with the hippocampal formation and olfactory bulb/cortex. The neocortex and various basal forebrain and diencephalic nuclei also showed significant hybridization, which increased with postnatal age. The cerebellum displayed alpha subunit mRNA primarily in the purkinje cell layer. Hybridization to beta subunit mRNA was also strongest in hippocampal and olfactory structures, followed by neocortex and basal forebrain nuclei. Unlike alpha subunit, hybridization to beta subunit mRNA in neocortex decreased with increasing age, and in cerebellum, was detectable in the granule as well as the purkinje cell layer. These results support previous findings indicating that the expression of the two CK-II subunits are differentially regulated during rat brain development.

463.7

TRANSFORMING GROWTH FACTOR BETA IN BRAIN TUMOR BIOLOGY. Harry T. Whelan and Harold L. Moses*. Depts. of Neurology and Cell Biology, Vanderbilt Univ., Nashville, TN 37232

We studied transforming growth factor beta (TGF-beta) in several clones of a virally induced canine glioma brain tumor cell line differing in their ability to produce brain tumors in vivo after intracerebral inoculation of the cells into dogs. In vitro colony formation and TGF-beta secretion by each clone correlated with in vivo tumorogenicity. In vivo tumor growth was documented pathologically in athymic "nude" mice (immunodeficient) as well as normal adult mongrel dogs. Only some of the brain tumor cell clones were able to produce brain tumors in normal dogs, however brain tumors were produced in all "nude" mice and all dogs pretreated with Cyclosporin.

Recent data (EMBO Jnl. 6:1633, '87) suggests that TGF-beta-1-or-2 is synthesized by human glioblastoma cells in vivo, contributing to impaired immunosurveillance and allowing brain tumor growth. We thus performed Northern blot hybridization on each clone to quantify Poly A mRNA for TGF-beta-1 and beta-2. The in vivo tumorogenecity of each brain tumor cell clone correlated with the amount of mRNA for both TGF-beta-1 and beta-2.

We postulate that TGF-beta stimulates glioma cell growth and inhibits host antitumor immune surveillance.

Supported by American Cancer Society Clinical Oncology Career Development Award No. 87-118 and NIH BRSG Award No. RR-05424.

463.8

DIFFERENTIAL REGULATION OF G-PROTEIN mRNA BY ADRENALECTOMY AND CORTICOSTERONE TREATMENT IN RAT CEREBRAL CORTEX. N. Saito*, R.S. Duman, X. Guitart*, E.J. Nestler, and J.F. Tallman. Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Previous studies have demonstrated that corticosterone (CORT) or adrenalectomy (ADX) alters the adrenergic receptor-coupled cyclic AMP generating system in the brain. The biochemical alterations responsible for these changes could involve one or all of the components of this system, which include the receptors, guanine nucleotide binding proteins (Gsα, Goα, Giα and Gβ), and the catalytic unit of adenylate cyclase. In the present study, we examined the influence of ADX and CORT on G-proteins by measuring mRNA levels for Gsα, Goα, Giα and Gβ by hybridization blot analysis using specific cDNA clones for each subunit. In addition, the total amount of Gsα, Goα and Gβ subunits was determined by immunoblotting analysis.

Giα mRNA levels in cerebral cortex were increased by ADX, and CORT replacement reversed this effect; in addition, chronic CORT administration alone decreased Giα mRNA levels. Gsα mRNA levels were increased by chronic CORT and a corresponding increase in Gsα immunolabeling was also observed; in addition, ADX tended to decrease Gsα mRNA levels. Gβ mRNA levels also tended to increase after CORT, while Goα mRNA levels and immunoreactivity were not altered by ADX or CORT treatment.

The results represent the first demonstration of differential regulation of G-protein mRNA levels and immunolabeling in the nervous system. These alterations of G-protein subunits may in part account for the influence of glucocorticoids on the adrenergic receptor-coupled cyclic AMP system in brain.

463.9

MOLECULAR CLONING OF THE HUMAN SUBSTANTIA INNOMINATA: DETERMINATION OF BRAIN REGIONALLY EXPRESSED mRNAs. B. E. Boyes*, D. G. Walker*, P. L. McGeer and E. G. McGeer. (SPON: J. R. O'Kusky) Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

In the primate brain the substantia innominata (SI) contains a group of magnocellular neurons, the majority of which are cholinergic. As such, this brain region represents a favourable source of material to characterize the genes expressed by these central cholinergic neurons. In order to examine gene expression in this region, a recombinant cDNA library has been prepared in the plasmid vector pTZ18 using SI polyadenylated RNA obtained from a neurologically normal individual. The small amount of SI RNA that can be obtained is a major difficulty in differential library screening. To identify regionally specified expressed genes, the SI library was initially screened with cerebellar cDNA. Clones which did not generate a hybridization signal with highly labelled cerebellar cDNA were further investigated by using the cDNA insert for brain regional hybridization analysis using RNA prepared from human SI, cerebellum, neocortex, corpus callosum and caudate nucleus. SI clones have been identified which correspond to genes differentially expressed in these brain regions. The sequence and cellular localization of the corresponding mRNAs are under investigation.

Supported by a grant from the ADRDA, Inc., Chicago. Brain tissues were obtained from the Alzheimer's Program, which is supported by the MRC of Canada and the B.C. Medical Services Foundation.

463.10

PHORBOL ESTERS STIMULATE APPEARANCE OF ASYMMETRIC ACETYLCHOLINESTERASE IN TTX-TREATED QUAIL MUSCLE CULTURES. C. Fernandez-Valle* and R.L. Rotundo. Dept. of Anatomy and Cell Biology, Univ. of Miami, Sch. of Med., Miami, Fl 33101

The major acetylcholinesterase (AChE) forms synthesized by tissue-cultured quail muscle are globular monomers, dimers and tetramers and asymmetric molecules, composed of three tetramers covalently linked to a collagen-like tail. Inhibition of spontaneous muscle contraction with tetrodotoxin (TTX) prevents appearance of asymmetric AChE, the predominant enzyme form at the neuromuscular junction. This does not occur via changes in synthesis, activation or degradation of newly-synthesized AChE polypeptide chains, but rather through a 2-fold increase in the fraction of total AChE activity secreted by non-contracting muscle cultures. This is suggestive of a block in assembly of tetramers with collagen-like tail subunits in the golgi apparatus. We have found that activation of protein kinase C with phorbol esters stimulates the appearance of asymmetric AChE in TTX-treated cultures. Inhibitors of PIP2 hydrolysis decrease total AChE activity to levels observed in TTX-treated cultures without affecting contractile activity. These studies suggest that regulation of synaptic components may occur through activation of second messengers in response to membrane depolarization rather than contractile activity per se. This work was supported by grants from the MDA and NIH to RLR; CFV is a State of Florida Pre-doctoral Fellow.

463.11

EXPRESSION OF MULTIPLE ACETYLCHOLINESTERASE TRANSCRIPTS IN QUAIL MYOTUBES IS REGULATED BY MUSCLE ACTIVITY. W. R. Randall*, C. Fernandez-Valle*, and R. L. Rotundo (SPON: A. Boyne). Department of Anatomy and Cell Biology, Univ. of Miami School of Medicine, Miami, Florida 33101.

Multiple oligomeric forms of acetylcholinesterase (AChE) expressed in avian and Torpedo nerves and muscle are encoded by a single gene. Using AChE-1 Torpedo cDNA as a probe (Schumacher et al., Nature 310: 407, 1986) we have isolated cDNA clones encoding quail AChE catalytic subunits. The identity of two cDNA clones was confirmed by DNA sequence analysis. Comparison of deduced amino acid sequences of Torpedo and quail AChEs so far obtained shows greater than 50% identity. One cDNA >4.5 kb is large enough to encode the entire open reading frame. Northern blot analysis of quail brain and muscle poly A+ RNA indicates the presence of multiple AChE transcripts in the range of 4.8 to 6 kb. Quail myoblasts express several AChE transcripts and synthesize membrane-bound and secreted AChE forms. The levels of AChE mRNAs, as well as rates of AChE translation, increase following myoblast fusion and subsequently decrease with the onset of spontaneous muscle contraction. Treatment of spontaneously contracting myotubes with tetrodotoxin results in a large increase in AChE mRNA. These studies show that expression of the AChE gene is developmentally regulated as well as under control by the activity state of the muscle. Supported by grants from the NIH and MDA to RLR.

463.12

cDNA CLONING OF A NOVEL NEURAL NICOTINIC RECEPTOR SUBUNIT FROM GOLDFISH RETINA. K.A. Cauley*, B.W. Agranoff and D. Goldman (SPON: K.A. Frey). Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

A way of producing changes at a synapse is to alter the properties or levels of the relevant neurotransmitter receptors. At the neuromuscular junction synaptic activity transcriptionally regulates the abundance of the nicotinic acetylcholine receptors (nAChRs). We are examining whether similar mechanisms exist for regulating nAChRs in the CNS, using the goldfish retina and regenerating optic nerve as a model system. We have screened a cDNA library prepared from RNA isolated from goldfish retina and have identified a novel nAChR subunit (GRAB-1). DNA sequence analysis shows GRAB-1 to encode a protein with structural features common to all nAChR subunits. We believe this clone is a non-alpha like subunit because it lacks the cysteines at positions 192 and 193 that are thought to constitute part of the ACh binding site in the alpha subunit. RNA blots show that GRAB-1 is expressed in the retina. S1 nuclease protection experiments indicate the presence of a second retinal mRNA identical to GRAB-1 for approximately 800 bases. In situ hybridization is being used to determine which cells in the retina are expressing this gene. (Supported by NEI Grant EY 05947.)

463.13

Appearance of a nAchR RNA Gradient Following Skeletal Muscle Denervation. J. Staple* and D. Goldman MHRI and Dept. of Biochemistry, University of Michigan, Ann Arbor MI 48109.

The neuromuscular junction (NMJ) is an excellent model system to study neural effects on synaptic organization. In adult innervated skeletal muscle, nicotinic acetylcholine receptors (nAchRs) and their RNAs are localized to the NMJ (Fontaine et al, 1988). At early times following muscle denervation a transient gradient of nAchRs exists, with receptor levels being highest in extrajunctional regions closest to the endplate (Levitt-Gilmour and Salpeter, 1986). This distribution may result from a gradient of nAchR RNA in denervated muscle.

The level of nAchR RNA in junctional and extrajunctional regions of the muscle fiber was determined by Northern blot analysis. Rat soleus muscles were denervated by cutting the sciatic nerve. At various times following denervation, muscles were removed and cut into thirds, one junctional and two extrajunctional pieces. RNA was isolated from these divided muscles and nAchR RNA levels were determined. At early times following muscle denervation we find higher levels of nAchR α, β, γ, and δ subunit RNAs in junctional versus extrajunctional regions of the muscle.

In situ hybridization was used to map the distribution of nAchR RNAs in the muscle fiber after denervation. The in situ results are consistent with those found by Northern blot analysis. At early times after muscle denervation, nAchR RNA is concentrated in the junctional region of the muscle fiber. Later, nAchR RNA levels increase in the extrajunctional regions of the muscle more distant from the endplate.

PAIN PATHWAYS: TRIGEMINAL SYSTEM

464.1

MORPHOLOGY AND PROJECTIONS OF INTRACELLULARLY LABELLED BRAINSTEM TRIGEMINAL VASCULAR CONVERGENCE NEURONS. A. Strassman, S. Potrebic*, M. Moskowitz, and R. Maciewicz. Pain Physiology Lab, Mass Gen Hosp, Boston MA 02114.

Brainstem trigeminal vascular convergence (TVC) neurons receive an excitatory, nociceptive input from cranial blood vessels as well as the periorbital skin or cornea. TVC neurons that responded to electrical stimulation of the superior sagittal sinus or middle meningeal artery as well as the cornea were identified with intracellular recording and then labelled with HRP. TVC cell somas were localized to the lateral part of lamina V in the rostral trigeminal nucleus caudalis; this distribution extended anteriorly into nucleus interpolaris, and ventrally into the trigeminal extension of the lateral cervical nucleus. Labelled neurons were typically either fusiform, with the soma and dendrites oriented along a ventromedial to dorsolateral axis, or multipolar in shape. Two patterns of TVC axonal projections were observed. The first type ascended through the spinal trigeminal complex, contributing multiple collaterals with terminations in lamina V and the adjacent medullary reticular formation. The second axon type crossed the midline to ascend in the trigeminothalamic tract. Before crossing the midline, some such axons collateralized in the dorsomedial reticular formation at the borders of the vagal and solitary nuclei. This pattern of terminations supports a role for TVC neurons in both sensory and autonomic cerebrovascular function.

464.2

MORPHOLOGICAL CHARACTERISTICS OF PHYSIOLOGICALLY DEFINED SINGLE TRIGEMINAL (V) BRAINSTEM NEURONES IN CATS. A. Yoshida*, E. Shohara*, J.G. Broton, J.O. Dostrovsky, Y. Shigenaga* and B.J. Sessle (SPON: T. Tashiro) Facs. Dentistry and Medicine, Univ. Toronto, Toronto, M5G 1G6 Canada and Hiroshima Univ., Japan.

To determine the morphological features of functionally identified V neurones, HRP-filled micropipettes were used to record and characterize the physiological properties of single neurones within V subnucleus oralis and adjacent brainstem regions. HRP was then injected intracellularly and the brainstem perfused. The 8 labelled oralis neurones had a mechanoreceptive field localized within one V division and short-latency (2-6 ms) electrically evoked inputs from skin, oral mucosa, or tooth pulp. Despite the restricted physiologically defined input, these neurones had an extensive dendritic tree: it had a mediolateral and dorsoventral span of 0.8-1.3 mm, and extended beyond the borders of the subnucleus. The thick stem dendrites averaged 6 in number and 7.8 μm in diameter and dendritic spines were abundant on the distal dendrites. The average soma diameter was 41 μm, and the axons and axon collaterals of many of these neurones could be traced out of the subnucleus to adjacent areas such as the V motor nucleus. Many of these features contrasted with those of functionally identified motoneurones labelled in the V (n=12) and VII (n=10) motor nuclei. (Supported by Can. MRC)

464.3

STIMULATION OF CRANIAL VESSELS ACTIVATES NEURONS IN THE THALAMUS OF THE CAT. A.S.Zagami*, G.A.Lambert* and J.W.Lance* (SPON: D.Tracey) Dept. of Medicine, University of New South Wales, NSW 2031, Australia.

Neurons in the ventroposteromedial (VPM) nucleus of the cat have previously been shown to be activated by noxious stimulation of orofacial structures. We have reported that electrical stimulation of the sagittal sinus (SS) and middle meningeal artery (MMA) activates neurons in VPM (Zagami,A.S. et al., Cephalalgia, 7:7-9, 1987) as have another group (Davis,K. and Dostrovsky,J., Pain, 4:S264, 1987). We now report that chemical and mechanical as well as electrical stimulation of cranial vessels activates neurons in the VPM as well as in other parts of the thalamus. The SS and MMA were stimulated electrically (40-120V,250us,0.2Hz) in chloralose anesthetized cats and single unit activity was recorded in the thalamus using glass coated tungsten electrodes. In some cases traction on the vessels was used to test for mechanical activation while in others bradykinin (BK) (10-4 to 10-5M) was applied locally. Electrical stimulation activated 44 units of which 23 were in VPM. Fifteen of these responded to SS stimulation, 5 to MMA and 3 to both. The mean latencies to SS and MMA stimulation were 18.4±2.1ms and 15.3±3ms respectively. Nine out of 10 SS linked units had receptive fields (RFs) usually periorbital and wide dynamic range in character. The mean latency for electrical stimulation of these RFs was 13.6±3ms. Traction on the SS and/or the MMA activated 4/4 units while BK was also effective in the units tested. Twelve units with a mean latency of 14.4±3.7ms to SS and/or MMA stimulation were found in the region of the posterior group of the thalamus. Some of these had facial RFs but they were often broad and sometimes bilateral. Seven responsive units were located in the zona incerta while another two were in the intralaminar complex.

464.4

SINGLE UNIT RESPONSES OF INFERIOR DENTAL NERVE TO THERMAL STIMULATION OF THE HUMAN TEETH :CORRELATION BETWEEN ELECTROPHYSIOLOGICAL DATA AND SUBJECTIVE PERCEPTION. K.IWATA*, K.TODA*, Y.TSUBOI*,J.YAGI* and R.SUMINO*(SPON:O.HIKOSAKA).Dept. Physiol. Nihon Univ. Sch. Dent. 1-8-13 Kandasurugadai, Chiyoda-KU, Tokyo 101, Japan.

Previous studies in animals have shown that thermal stimulation of teeth evokes responses in dental nerves but it is not known that the animals really receive pain sensation. However, anatomical studies reported that there were different populations in the intra-dental nerve fibers between animals and humans, suggesting that the sensory mechanisms of tooth pain to thermal stimulation were different between them. Therefore, these proposed us to study the correlation between the intra-dental responses to thermal stimulation of the tooth and the perceived sensation in man.

Young adult men were used in this study. Single fiber discharges were recorded from the inferior dental nerve with the enamel coated tungsten micro-electrodes inserted through the mental foramen. Majority of cold sensitive and heat sensitive fibers responded with bi-phasic firing (phasic and static responses) and with long lasting after discharges following thermal stimulation of the lower incisor. Almost all fibers were categolized as the slowly conducting Ao and C fibers. During the thermal stimulation of the tooth, firing rate of the fibers were correlated to the ratings of the subjective sensation. Subjects did not feel any kinds of sensation after the cessation of thermal stimuli, although, after discharges continued.

464.5

COMPARATIVE CENTRAL REPRESENTATION OF MAXILLARY AND MANDIBULAR DENTAL STRUCTURES. M.A. Henry and L.E. Westrum. Depts. of Neurol. Surg., Biol. Struct., and Restorative Dent., Univ. of Wash., Seattle, WA 98195.

The large pulpal chambers and apical foramina of permanent but immature dentition allow maximal HRP labeling of pulpal fibers and easy spread to periodontal ligament. Here we report a study of the central somatotopic organization of maxillary (Mx) as compared to mandibular dental structures (Md) following HRP injections into either a maxillary or a mandibular cuspid of a 30-week-old cat. LM shows considerable overlap between Mx and Md within dorsomedial main sensory nucleus (MSN). All spinal nuclei show a slight overlap. In pars oralis (PO) Md is most dorsal and Mx just ventral to it. In pars interpolaris (PI) Md and Mx have the same dorsal-ventral relationship as PO but occupy a more medio-ventral part of the nucleus. Pars caudalis (PC) shows a mediolateral pattern in dorsal nuclear regions with Md more medial. A pathway for primary afferent crossing to the contralateral side is demonstrated near obex and a ventral projection is seen in PI/PC. Large fibers traverse MSN/PO, enter the mesencephalic (Mes V) tract and lead to labeled cells in the Mes V nucleus. Other labeled fibers located in the motor tract show some terminal arbors. The findings show clearly separate but overlapping patterns of Mx and Md, a crossing pathway and a periobex ventral termination, (Supported by NIH Grants DE00219 and DE04942. LEW is an affiliate of CDMRC.)

464.6

CALCITONIN GENE - RELATED PEPTIDE LIKE IMMUNOREACTIVITY IN FELINE TRIGEMINAL NUCLEI. L.R. Johnson*[1], M.A. Henry[2], L.E. Westrum[2] and N.A. Nousek-Goebl*[2] (SPON: J. Loeser). [1]University of Colorado, Denver, CO 80262, [2]University of Washington, Seattle, WA 98195.

Recent studies have localized calcitonin gene - related peptide like immunoreactivity (CGRP-LI) in a variety of peripheral structures that receive trigeminal afferent innervation. The present study examines CGRP-LI in the brain stem trigeminal nuclear complex. CGRP-LI occurs in the trigeminal ganglion and all central trigeminal nuclei except the mesencephalic nucleus. In the main sensory nucleus (MSN) CGRP fibers in the ascending tract send a dense projection to the dorsomedial MSN and a sparse one to the ventrolateral MSN. CGRP positive fibers are present in the descending trigeminal tract giving off axons to all the spinal trigeminal nuclei. Pars oralis has the heaviest labeling dorsally, while pars interpolaris shows more ventral accumulations of CGRP-LI. Dense label occurs in layers I and II of the medullary dorsal horn with fibers leading to deep pockets of label in laminae V and VI. Labeled fibers cross the brain stem dorsal to the central canal. Some of these patterns are strikingly consistent with those described for dental projections. LEW is an affiliate of the CDMRC. Supported by NIH Grants DE04942, DE00219 and NS09678.

464.7

EFFECTS OF ANAESTHESIA ON RESPONSES OF TRIGEMINAL BRAINSTEM NEURONES TO TOOTH-PULP STIMULATION IN THE CAT. F.M.Boissonade* and B.Matthews, Dept. of Physiol., Univ. of Bristol, England.

Recordings of responses to stimulation of tooth-pulp and other oro-facial tissues have been made from neurones in the trigeminal main sensory and spinal (mainly pars oralis) nuclei of conscious, unrestrained cats and from the same neurones when the animals were lightly anaesthetized with alphaxalone/alphadolone (4 mg/kg, I.V.). The animals were prepared in an initial operation under general anaesthesia. A titanium chamber, which incorporated a mounting ring into which a miniature micromanipulator could be mounted, was positioned stereotaxically over a craniotomy in the posterior cranial fossa. The dura was covered with silicone elastomer and the craniotomy covered with a titanium plate. A connector block with a miniature 9-way socket and venous injection port was fixed to the skull over the frontal sinus. Leads were passed subcutaneously from this block to Ag/AgCl fillings in the right canine teeth for electrical stimulation of tooth-pulp, and to the right digastric muscle for recording its E.M.G. A cannula from the injection port was inserted into the anterior facial vein. Recordings were made with glass-insulated tungsten electrodes and began three days after the initial operation.

In a conscious animal, single tooth pulp stimuli of 0.1ms duration and up to 50X digastric reflex threshold produced no aversive behaviour. Short (~3ms) and long (~25ms) latency neuronal discharges were evoked. Light anaesthesia reduced the number of impulses in both responses, particularly the second, but had little effect on the threshold of the first. It affected responses to other forms of stimuli in only a small proportion of neurones. It reduced spontaneous activity of neurones and also the amplitude of the digastric reflex.

464.8

RESPONSE PROPERTIES OF TOOTH PULP-DRIVEN CELLS IN THE SPINAL TRIGEMINAL NUCLEUS ORALIS OF THE CAT. A.Pertovaara, T.Huopaniemi*, E.Jyväsjärvi*, S.Carlson* and F.Lindroos*. Dept.Physiol.,Univ. of Helsinki, Finland.

Tooth pulp-driven cells were recorded in the trigeminal nucleus oralis of the cat. The thresholds to monopolar electric pulses were determined using a constant current stimulator. The most sensitive oralis cells had lower thresholds and shorter latencies than the respective caudalis or interpolaris cells. In general, the thresholds were lower and the strength-duration curves flatter than those depicting liminal dental pain in man, but similar to those depicting liminal jaw reflexes. The relationship between stimulus intensity and response magnitude could well be described by power functions. The thresholds were not elevated during a noxious tail pinch. The converging input from the skin was from low-threshold mechanoreceptors. The results indicate that there are significant differences in the response properties of cells of the different spinal trigeminal subnuclei. Human pain thresholds cannot be explained by the liminal response properties of the most sensitive oralis cells but they might be important in the mediation of liminal reflex events.

464.9

IS TOOTH PULP REPRESENTED IN THE MESENCEPHALIC TRIGEMINAL NUCLEUS? N. Amano*, K. Yoshino*, H. Matsuoka*, S. Andoh* and S. Kawagishi* (SPON: T. Sugimoto). Dept. Oral Neuroscience, Kyushu Dental College, Kitakyushu, 803 Japan.

Several physiological and histological studies have shown the presence of a substantial proportion of pulpal afferent fibers with conduction velocities and diameters greater than the Aδ range in the tooth pulp of mammals including man. The purpose of this study is to examine a possible dual innervation of tooth pulp from the mesencephalic trigeminal nucleus (MTN) and trigeminal ganglion (TG) in the cat using retrograde transport of horseradish peroxidase (HRP). Great care was taken to avoid a leakage of HRP through the apical foramen into periodontal tissue and to minimize inhibition of axonal transport of HRP due to acute pulpitis in the tooth applied with HRP. Pulp chamber of each left lower canine of 8 adult cats was opened with a dental air turbine under water cooling. Following partial pulpotomy, 1–2 µl of 30% HRP in saline soaked into gelfoam was placed in each pulp chamber. The openings were sealed with dental glass-ionomer cement. Immediately after the surgery and throughout the subsequent 3-day survival period, 6 cats were subjected to systemic administration of anti-inflammatory drug, prednisolone, whereas the remaining 2 control cats were not. Histological sections were processed according to TMB technique by Mesulam. Only in 6 prednisolone-treated cats many HRP-labeled neurons were observed in both MTN and TG on the side ipsilateral to the HRP application.

SUBCORTICAL SOMATOSENSORY PATHWAYS: TRIGEMINAL SYSTEM

465.1

CHARACTERIZATION OF TRIGEMINOTECTAL AFFERENTS IN THE SUPERIOR COLLICULUS OF THE RAT. E. Gregory and W. C. Hall. Dept. of Neurobiology, Duke Univ., Durham, NC 27710.

The intermediate grey layer of the superior colliculus in the rat receives somatosensory projections from the spinal trigeminal nucleus. The present experiments were designed to characterize the terminations of this pathway as a step toward determining their contribution to the output of the various efferent pathways of this layer. We placed iontophoretic injections of 2.5% biotinylated PHA-L (Vector) in subnucleus interpolaris of the spinal trigeminal nucleus of three Long Evans rats. Six days after surgery the rats were perfused; the tissue was then processed for PHA-L immunocytochemistry and reacted in diaminobenzidene. For electron microscopy, tissue was embedded and areas containing terminations were thin-sectioned. PHA-L positive fibers are restricted to the rostral two-thirds of the superior colliculus. In the intermediate grey layer (Weiner's nomenclature), the label appears "patchy" because of variations in terminal and fiber density. The labeled axons are numerous laterally, and become sparse medially. Most axons are fine and highly branched. Many are beaded, giving the appearance of boutons en passant. Electron microscopic examination reveals that labeled terminals are filled with densely packed, small round vesicles and contact dendrites. Experiments are in progress to identify the target cells of these axons. Supported by Grant #BNS-86-07060.

465.2

WHAT'S IN A PATCH? EXAMINATION OF THE TRIGEMINAL PROJECTION TO THE SUPERIOR COLLICULUS WITH PHASEOLUS VULGARIS LEUCO-AGGLUTININ AND SINGLE FIBER LABELLING. R.W. Rhoades, R.D. Mooney, N.L. Chiaia, M.M. Nikoletseas, and W.H. Rohrer, Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Anterograde tracing with a variety of methods has demonstrated that the vast majority of the inputs to the deep laminae of the mammalian superior colliculus (SC) terminate in a discontinuous or "patchlike" fashion. There has been some disagreement as to whether these patches represent terminal arbors or bundles of preterminal axons. Anterograde tracing of the projections to the SC from trigeminal principalis (PrV) and subnucleus interpolaris (SpVi) with Phaseolus vulgaris leuco-agglutinin has allowed us to demonstrate that the patches observed with other methods are indeed terminal arborizations. Labelling of individual fibers by means of intra-axonal injection of horseradish peroxidase demonstrated further that individual trigeminotectal axons contributed to several patches across the mediolateral extent of the stratum album intermedium. We also noted that the SC projection from PrV consistently had several fibers which recrossed the midline in the SC commissure to give rise to patchy terminal arbors in the SC ipsilateral to the injection site. Supported by BNS 85-00142, BNS 85-17537, EY 04170, DE 07734.

465.3

MORPHOMETRY OF BARREL PRECURSOR PRIMARY AFFERENTS IN TRIGEMINAL NUCLEUS PRINCIPALIS. W.M. Panneton, W.E. Renehan, J. Golden* & M.F. Jacquin. Anat. & Neurobiol., St. Louis Univ. Sch. Med., MO 63104 & Anat., Univ. Louisville, KY 40292.

A neuron reconstruction system (Eutectic Elect.) was used to quantify the 3-dimensional structure of 2 electrophysiologically characterized, HRP-labeled, primary afferent fibers with collaterals in the "barrel" region of rat principalis (PrV). Each responded phasically to deflection of 1 mystacial whisker and gave rise to 6 collaterals with arbors distributed discontinuously in topographically appropriate regions of PrV. Collaterals branched 19.8±7.8 times. En passant and terminal swellings were usually 1.5um or less and were distributed unequally across branch order with most on 6th order segments or greater. Arbor envelopes of each collateral had transverse areas of 15333±14626um2 and volumes of 353380±526815um3. Arbor limits were 159±69, 173±132 and 72±38um in X,Y,Z respectively. However, one of these collaterals was unusual in having 2 second-order branches which arborized in nonoverlapping regions of PrV. Excluding this collateral, the remaining 11 collaterals had transverse areas of 11185±5187um2 and volumes of 198052 ±115077um3, while arbor limits were 154±70, 136±52 and 63±26um in X,Y,Z respectively. Thus, most of the collateral arbors had a similar geometry to that of single whisker sensitive, thalamic-projecting cells in PrV (Golden & Jacquin, this volume), but the arbors most often are larger and could contain completely one of these PrV cells. Support: NIH Grants DE07662, DE07734.

465.4

INTERSUBNUCLEAR PROJECTIONS IN THE RAT TRIGEMINAL BRAINSTEM COMPLEX. P.A. Young, R.W. Rhoades, N.L. Chiaia, P.R. Hess* & M.F. Jacquin. Anat. & Neurobiol., St. Louis Univ. Sch. Med., MO 63104 & Anat., Med. Coll. Ohio, Toledo, OH 43699.

Prior retrograde tracing and Golgi studies, primarily in cat, indicate a complex web of intersubnuclear connections within the trigeminal (V) brainstem complex. In rat, HRP-stained local circuit neurons, and some projection cells, in subnuclei caudalis, interpolaris, and oralis, have been shown to project to rostral and caudal subnuclei. In the present study, anterograde transport of Phaseolus vulgaris leukoagglutinin was used to visualize the projections and morphologies of V intersubnuclear axons. Injections restricted to caudalis (N=6) resulted in dense terminal labeling in each of the more rostral ipsilateral V subnuclei and cervical dorsal horn, and sparse label in contralateral caudalis. Interpolaris (N=4) projected heavily to ipsilateral caudalis, oralis and principalis. Principalis (N=4), on the other hand, had only a sparse projection to each of the caudal ipsilateral subnuclei. The smaller of the above injections showed axon endings in regions topographically matching their sites of origin. Individual collaterals could be reconstructed in most cases. Their morphologies were as previously described for single HRP-stained cells. Axons traveled within the deep bundles of the V brainstem complex, the V spinal tract and reticular formation. Most collaterals gave rise to circumscribed and highly branched arbors with a large number of terminal and en passant boutons. Support: NIH DE07662, DE07734, NSF BNS8515737.

465.5

MORPHOMETRY OF BARREL PRECURSOR CELLS IN TRIGEMINAL NUCLEUS PRINCIPALIS. J. Golden* & M.F. Jacquin (SPON: K. Smith). Anat. & Neurobiol., St. Louis Univ. Sch. Med., MO 63104.

A neuron reconstruction system (Eutectic Elect.) was used to quantify the 3-dimensional structure of 5 HRP-labeled cells in the "barrel" region of rat principalis. Each responded phasically to 1 mystacial whisker and projected only to thalamus. Mean latencies to V ganglion and thalamic shocks were 1.1 and 1.4 ms. Small somata gave rise to 5.6±2.3 proximal dendrites which first branched 23±14um from the soma. Similar lengths separated 19±5 subsequent branch points. All dendritic branching occurred within 100um of the soma, with the largest # of branch points between 20-30 um. 64±21 dendritic appendages (swellings, spines) were equally distributed across branch order and were most numerous on 0.4-1.2um thick branches. Total dendritic length, surface area and volume were 880±181um, 2740±597um2 and 925 ±293um3, respectively. Dendritic tree envelopes had transverse areas of 3225±863um2 and volumes of 29727±10473um3. Each tree was polarized, spanning no greater than a hemisphere around the soma; however, there was no reliable direction of polarity. Centers of dendritic area, relative to somata, were 4±23, -2±8 and -5±11um in X,Y,Z respectively. Tree limits were 68±14, 95±48 and 91±29um in X,Y,Z respectively. These small and dense dendritic trees have similar shapes to that of whisker afferent collaterals in principalis (Panneton et al., Soc. Neurosci. Abst., this volume). Their matching shapes may explain receptive field size in single-whisker principalis cells. Support:DE07662,DE07734.

465.6

CELL FORM AND FUNCTION IN TRIGEMINAL NUCLEUS PRINCIPALIS. K.L. Rossner, W.M. Panneton & M.F. Jacquin. Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. Med., MO 63104.

Intracellular recording, electrical stimulation, receptive field mapping, and HRP injection techniques were used to study principalis cells in rat. 80 cells provided physiological data. They responded within 1.2±0.2 ms of trigeminal ganglion shocks and 69% were antidromically activated by thalamic shocks (1.3±0.5 ms latencies). 69% were whisker-sensitive; of these, 80% were responsive to 1 whisker (mean=1.45, range=1-8), 29% were slowly adapting, 40% were direction sensitive, 35% were spontaneously active, 2% had an inhibitory surround, and 4% also discharged to guard hairs. The rest responded to guard hairs (13%), skin (5%), teeth (1%), or nociceptors (8%), and 3% were unresponsive. 17 cells were HRP-stained. Thalamic-projecting cells with 1 whisker, guard hair, or skin receptive fields (N=12) had small somata and dendrites which extended only a short distance from the soma, where they branched extensively. Spines were rare, yet swellings were common. Axons never branched locally. Within this group, slowly adapting cells tended to have bigger dendritic trees with more swellings. As a group, however, these 12 cells had very different morphologies from those of 1 nociceptive, and 2 multi-whisker projection cells, and 2 local circuit cells. Larger somata gave rise to expansive and spiny dendritic trees, often with local, intersubnuclear, and reticular axon collaterals. These data are suggestive of structure-function correlations in principalis. Support: NIH DE07662, DE07734.

465.7

CELL FORM AND FUNCTION IN TRIGEMINAL SUBNUCLEUS ORALIS. N. Hobart*, W.M. Panneton & M.F. Jacquin (SPON: N. Connors). Anat. & Neurobiol., St. Louis Univ. Sch. Med., MO 63104

Intracellular recording, electrical stimulation, receptive field mapping, and HRP injection techniques were used to study form-function correlations in oralis of the rat. Of 15 labeled cells, 4 were local circuit neurons responsive to either an incisor, guard hairs, 1 vibrissa, or deep pressure. Their somadendritic structures most closely resembled those of V-thalamic cells in subnucleus interpolaris (Jacquin et al., Exp. Br. Res.: 61, '86); their axons had intersubnuclear and local collaterals. Thalamic- (N=6) and cerebellar-projecting (N=2) cells had response properties and morphologies which were similar to interpolaris cells with equivalent projections. 2 cells projected to spinal cord, as well as other V subnuclei; one responded to strong pressure applied to an incisor; the other was 1 whisker sensitive. Their morphologies did not differ from other oralis and interpolaris projection neurons. The remaining cell had 2 axons, one projecting to thalamus, the other to spinal cord. It responded to 15 vibrissae, guard hairs and glabrous skin. Its somadendritic morphology was similar to that of other oralis projection neurons.

The extensive dendritic trees, local and long-range axon branching, multi-whisker convergence, and functional diversity of oralis cells approximates that observed in interpolaris. Such anatomical and physiological properties are rarely seen, however, in subnucleus principalis. Support: NIH grants DE07662 and DE07734.

465.8

INNERVATION OF THE INNER CONICAL BODY IN THE RAT VIBRISSAL FOLLICLE-SINUS COMPLEX (F-SC). T.M.Mosconi,* F.L.Rice, H.Aldskogius, and J. Arvidsson. Anatomy Dept., Albany Medical College, Albany, NY 12208; Anatomy Dept., Karolinska Institute, Stockholm, Sweden.

The inner conical body (ICB) in a mystacial vibrissal F-SC is heavily innervated only in species that whisk (Rice et al., JCN 252:154). As seen in LM silver preparations of the adult rat ICB, numerous receptors are parallel to each other and encircle the vibrissal follicle in a plane perpendicular to the hair shaft. One of our goals was to determine the ultrastructure of the adult rat ICB innervation by standard TEM of thin sections and High Voltage EM of thick (0.25-0.5um) sections. Numerous endings were observed: many nearly free of surrounding structure (free nerve endings?) of which one novel type resembled grapeshot; many embedded in thick collagen matrices (?); some affiliated with collagen fibers in a "septal cell" capsule (Ruffini endings?); and a few others flattened between two satellite cells (lanceolate endings?). Another goal was to evaluate some neuropeptide LM immunoreactivity (IR) within the ICB. Numerous, but clearly not all processes expressed substance P- and CGRP-like IR. No ICB processes expressed somatostatin-, VIP-, or NPY-like IR. (Support: Swedish Medical Research Council and NIH PHS RR01219 to Drs. Donald Parsons and Min Song, NY State HVEM Facility)

465.9

PRINCIPALIS- OR PARABRACHIAL-PROJECTING SPINAL TRIGEMINAL NEURONS DO NOT STAIN FOR GABA OR GAD. J.H. Haring & M.F. Jacquin. Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Most low-threshold, mechanoreceptive local circuit neurons in rat spinal trigeminal (SpV) subnuclei interpolaris and caudalis have collaterals which end in more rostral subnucleus principalis (PrV). It has been hypothesized that these cells have GABA-ergic processes which serve to gate transmission of V primary afferent inputs to projection neurons in SpV and PrV. Retrograde transport of diamidino yellow (DY) and immunohistochemical double-labeling procedures were used to determine if pontine-projecting SpV cells stain positively for GAD or GABA. As expected, large bilateral injections of DY into rostral and lateral pons (N=10), inclusive of PrV and parabrachial nuclei, labeled large #'s of cells in each SpV subnucleus. Brainstems (2 colchicine pretreated) were subsequently reacted for cytoplasmic GAD or GABA immunoreactivity (Weinberg et al., Neurosci. Lett. 55:349, '85). A large # of cells throughout SpV were immunopositive (approx. 450, 200 and 160 cells per cross-section through caudalis, interpolaris and oralis, respectively), with the heaviest concentration in ventral interpolaris and laminae II of caudalis. However, none were double-labeled with DY. This result does not support the above-stated hypothesis, suggesting that SpV local circuit neurons with PrV collaterals are not GABA-ergic. These studies also indicate that parabrachial-projecting SpV cells are not GABA-ergic. Support: NIH DE07662, DE07734, NS25752.

466.1

PROGRESS ON THE CYCLIC ADENOSINE MONOPHOSPHATE RECEPTOR OF PARAMECIUM. J.L. Van Houten, J. Baez*, B. Cote*, J. Zhang*. Dept. of Zoology, Univ. of Vermont, Burlington, VT 05405

Cyclic AMP is an attractant stimulus to paramecia. As other attractants, it hyperpolarizes the cells and shows specific, saturable, albeit low affinity binding to whole cells, primarily on the cell body (not ciliary) membrane. A 48 Kd protein identified by affinity chromatography is a candidate for the cAMP receptor, as judged by its specificity for binding to the column. Although similar in molecular weight, it is not identical to the regulatory subunit of the Paramecium cAMP-dependent protein kinase on Western blots (antibody provided by M. Hochstrasser). In 2D electrophoresis, the 48 Kd protein is extreme in its pI (2.5). Con A-HRP does not appear to bind to the protein; however, it still may be a glycoprotein. Since the N terminus is blocked, microsequencing is being carried out on CNBr fragments. The sequence will be used to produce an oligonucleotide probe for clong the receptor gene. Polyclonal antibodies have been produced in New Zealand rabbits against gel slices containing the antigen. These antibodies recognize the antigen in ng amounts on slot blots and, therefore, are appropriate for screening an expression library of lambda gtll for the receptor gene. Supported by NSF.

466.2

FOLATE CHEMORECEPTOR MUTANT: ANALYSIS OF SURFACE MEMBRANE PROTEINS IN PARAMECIUM. J.M. Sasner, J.K. Isaksen, and J.L. Van Houten. Zoology Department, University of Vermont, Burlington, VT 05405.

Paramecium are attracted to folate by a chemokinetic mechanism. The cell body membrane binds folate specifically and saturably, and this binding correlates with the change in membrane potential that determines swimming behavior. We are trying to identify the receptor protein for folate by comparing the folate-binding proteins of membrane preparations of wild-type cells with those of mutants that do not normally bind or respond to folate.

We have developed a method to covalently crosslink folate to cells that specifically blocks folate chemoreception, and have developed an antibody that specifically detects the ligand. Preliminary data also shows that we can covalently crosslink iodinated folate to membrane preparations. We are using these techniques to identify membrane folate binding proteins and by comparison of wild-type and mutant folate crosslinked proteins, will identify the chemoreceptor protein.

Supported by NSF and Whitehall Foundation.

466.3

EXPRESSION OF NEURONAL ENZYMES AND CARBONIC ANHYDRASE IN CULTURED GLOMUS CELLS OF THE RAT CAROTID BODY. C.A. Nurse, L. Farraway* and C. Vollmer*. (SPON: J. Rowe). Dept of Biology, McMaster University, Hamilton, Ontario, L8S 4K1.

We are studying neurotransmitter and transduction mechanisms in a chemosensory organ, the rat carotid body, using dissociated cell cultures (see accompanying abstract, A. Stea and C.A. Nurse). Previous studies in this laboratory indicated that single (parenchymal) glomus (type I) cells in these cultures may express both catecholamine histofluorescence as well as acetylcholinesterase (Cell Tissue Res. 250: 21-27). We now show by the use of immunofluorescence techniques that these glomus cells selectively express both tyrosine hydroxylase and neuron specific enolase immunoreactivity. In addition, histochemical staining for carbonic anhydrase (CA) indicated a selective localization in glomus cells; staining was markedly enhanced following permeabilization of the cell membranes, suggesting a predominant intracellular localization, and was abolished by the CA-inhibitor acetazolamide (10 μM). Unstained cells included sustentacular (type II) cells and fibroblasts. This finding is important since CA-localization in the carotid body is controversial and bears on the precise formulation of the acidic hypothesis, proposed to explain the organ's response to increased blood CO_2 (and H^+) levels.

Supported by the Heart and Stroke Foundation of Ontario.

466.4

SURFACE HETEROGENEITY OF HUMAN OLFACTORY EPITHELIUM. M.I. Chuah and D.R. Zheng*. Dept. of Anatomy, Chinese University of Hong Kong, Hong Kong and Dept. of Anatomy, Jinan University, Guangzhou, China.

We have conducted scanning (SEM) and transmission electron microscopic (TEM) observations on the human olfactory epithelium (OE) of the nasal septum (NS) and superior concha (SC). Our findings show that the human OE surface, hitherto assumed to be uniform, exhibits considerable heterogeneity.

In the SC, the OE surface was highly ciliated, suggesting a high density of receptor cells. For the most part, the length of the cilia was uniform. Microvilli, indicating the presence of supporting cells, were generally present in small pockets. In SEM, dendrites were observed to give rise to large numbers of cilia, more numerous than previously estimated from TEM studies. However, the usual knob-like endings of the dendrites were not apparent.

In the NS, the distribution of ciliated and microvillous surfaces was more variable than that of the SC. Generally, the ciliated regions occupied a smaller area; and patches of cilia of different lengths were often present in close proximity. Some dendrites bore radiating arrangements of cilia, which were not observed in the SC. TEM observations of young receptor cells showed that many of them possessed cilia with expanded tips (primary cilium?). Ductal openings of Bowman's glands were more numerous in the NS.

466.5

NUMERICAL RELATIONS BETWEEN OLFACTORY RELAY ELEMENTS (RECEPTOR NEURONS, GLOMERULI AND MITRAL CELLS) IN THE CAT. Tia Simms* and E. Meisami (SPON: C.L. PROSSER). Dept of Physiology, Univ. Illinois, Urbana, IL 61801

The olfactory mucosa (OM) and bulb (OB) of adult cats were analysed quantitatively for the number of primary olfactory neurons (ON), the glomeruli (GL) and mitral cells (MC) using light microscopic, numerical and morphometric methods. Complete frontal series of 10 um whole sections, stained with H&E and Nissl were employed. The results indicate that the cat possesses, on each side of the nasal cavity, about 40 million ONs which converge onto about 40,000 MCs in each OB. This gives an ON:MC convergence ratio of about 1000:1, the same as in the macrosmatic rabbit. If this convergence ratio can be considered as a neural index of olfactory sensitivity, then the cat may be as sensitive as the rabbit. The number of GL was estimated as 10,000 if the mean glomerular diameter was taken as 70 um (i.e., mean diameter of all GL per section) or 3300 if the mean diameter was taken as 105 um (i.e., average of the largest GL diameters per section). The OB volume is about 50 cu mm and MC layer surface area about 50 sq mm, giving an average of 800 MCs/sq mm. Total OM surface area is 600 sq mm, 20% covering the septum, 80% the conchae; the surface density of the ONs being about 70,000/sq mm. The OM sheet is about 50 um thick and has 9 and 3 ONs for each basal and supporting cells respectively.

466.6

LIQUID ODORANTS DELIVERED TO THE VOMERONASAL ORGAN OF GARTER SNAKES INCREASE FIRING OF CELLS IN THE ACCESSORY OLFACTORY BULB. J. Inouchi, J. L. Kubie and M. Halpern. Dept. of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, Brooklyn, N. Y. 11203.

Most terrestrial vertebrates possess both main olfactory and vomeronasal systems with many features in common. There have been few studies using electrophysiological methods to characterize and compare the properties of these two systems. To examine the physiological properties of both systems in garter snakes (Thamnophis sirtalis parietalis), we first focused on recording electroolfactograms (EOGs) from the olfactory and vomeronasal epithelia. Using airborne odorants we were able to elicit EOG responses from the olfactory and vomeronasal epithelia although the responses from the vomeronasal epithelium were very small (less than 1 mv). It has been suggested that the vomeronasal system is normally stimulated by odorants delivered as liquids. In recordings from individually recorded accessory olfactory bulb neurons we found a variety of liquid odorants, including amyl acetate and earthworm wash, induced dramatic increases in neuronal activity. These results demonstrate that the vomeronasal system is sensitive to a variety of odorants and support the idea that under normal conditions effective stimuli arrive in a liquid medium.

Supported by NIH Grant NS11713.

466.7

EOG AMPLITUDE IS CORRELATED WITH ODOR-STIMULATED ADENYLATE CYCLASE ACTIVITY IN THE BULLFROG OLFACTORY EPITHELIUM. Graeme Lowe*, Tadashi Nakamura* and Geoffrey H. Gold. Monell Chemical Senses Center, Philadelphia, PA.

Recent work suggests that odor-stimulated adenylate cyclase (AC) activity mediates olfactory transduction in vertebrates (Pace et al., 1985; Shirley et al., 1986; Nakamura and Gold, 1987). However, stimulation of the AC by certain odorants is weak or undetectable, leading to the suggestion that the AC may not mediate transduction for all odorants (Sklar et al., 1986). An alternative explanation for these data is that differences in the magnitude of AC stimulation by various odorants may reflect differences in the number of receptor proteins or receptor cells sensitive to those odorants. If the latter hypothesis is correct, we expect the amplitude of the EOG to be correlated with the magnitude of AC stimulation by various odorants.

The EOG was recorded from excised bullfrog olfactory epithelia mounted in a perfusion chamber. Odors were applied to the apical side, dissolved in Ringer's solution at a concentration of 100 uM to approximate the conditions used in Sklar et al.'s cyclase assay. Of 35 odorants tested thus far, 31 odorants elicited a monophasic negative EOG, and 4 odorants elicited a multiphasic negative EOG. The multiphasic responses may contain contributions from currents unrelated to transduction, and therefore, were not included in the analysis below. The correlation coefficient relating the peak amplitude of the EOG to the magnitude of AC stimulation for each odorant was 0.87.

Our data show that the ability of odorants to stimulate adenylate cyclase is correlated with their ability to generate an EOG response. These results support the adenylate cyclase model of olfactory transduction.

466.8

RELATION BETWEEN NERVE FIBER TYPE AND AMILORIDE INHIBITION OF TASTE RESPONSES IN THE HAMSTER. M.E. Frank and T.P. Hettinger*. Department of BioStructure and Function, University of Connecticut Health Center, Farmington, CT 06032.

Current interest in the involvement of ion channels in taste reception and transduction is based in part on electrophysiological and behavioral effects of orally applied channel blockers in several vertebrate species. Recordings of integrated responses of the whole chorda tympani nerve have shown effects attributable to involvement of Na and K channels in taste stimulation by a variety of salts. We find in the hamster that physiologically defined classes of chorda tympani fibers respond differentially to lingual application of amiloride, a blocker of epithelial Na channels. N fibers (n = 16), those responding selectively to Na and Li salts, can be completely inhibited by 10 uM amiloride, while H fibers (n = 10), those showing a broad spectrum of response to salts and acids, are not affected. The partial inhibition seen in the whole nerve is explained by these observations. The segregation of amiloride effects according to fiber type shows that chemoreceptive mechanisms differ for the two fibers types that respond to NaCl. Amiloride presumably acts on sodium channels of the apical membrane of taste receptor cells. Thus, for N fibers, the molecular receptors for Na salts may be identical to Na channels. Receptor cell activation in this system could be achieved by direct depolarization without requiring a second messenger. Supported by NSF Grant BNS-8519638.

466.9

A SUBSET OF RAT PRIMARY OLFACTORY NEURONS AND GLOMERULI SHOWN BY ANTIBODY TO HUMAN PLACENTAL ANTIGEN COMMON TO ESTROGEN-BIOSYNTHETIC ORGANS. K. Shinoda[1]*, Y. Shiotani[1]*, J. Pearson[2] and Y. Osawa[3]*. 1. Osaka University Medical School, Japan, 2. NYU Medical Center, NY, 3. Medical Foundation of Buffalo, NY.

Primary olfactory neurons have not yet been extensively classified. A solitary, "modified glomerular complex" located caudally between the main and accessory olfactory bulbs is involved in "suckling behavior" response to nipple and amniotic fluid odors in rats. We now report localized immunoreactivity in frozen sections of paraformaldehyde fixed rat olfactory bulb using an antiserum to human placental antigen. The antiserum stains estrogen synthesizing organs (including placenta and ovary) and suppresses 73% of the activity of human placental P450 aromatase. A distinct subset of rat olfactory glomeruli forms a necklace-like pattern at the caudal end of the rat olfactory bulb and is associated with a subclass of primary olfactory receptors. Part of this subset of sensory neurons in the rat primary olfactory system appears identical with the glomerulus responsible for suckling behavior.

466.10

***CONCANAVALIN A* CYTOCHEMISTRY ON RAPIDLY-FROZEN RAT OLFACTORY EPITHELIAL SURFACES, FREEZE-ETCHED AND ROTARY-REPLICATED WITH TANTALUM/TUNGSTEN OR FREEZE-SUBSTITUTED AND EMBEDDED IN LOWICRYL K11M.** B. PH. M. Menco *Dept. of Neurobiology and Physiology, O.T. Hogan Hall, Northwestern University, Evanston, IL 60208.*

Tantalum/tungsten (Ta/W) freeze-etch replicas are considerably thinner, and hence contain more information than conventional platinum/carbon (Pt/C) replicas (1). Rat olfactory epithelial samples were incubated with the lectin Concanavalin A (Con A) conjugated to 5 nm colloidal gold particles at 0°C for 2 to 4 hours before cryo-fixation. Frozen specimens were then fractured at -140°C, etched for 30 minutes at -108°C and rotary-replicated (300-400 rpm) with Ta/W. The Ta/W replicas were, on average, about 0.2 nm to 0.3 nm thick, which is much thinner than the 2 nm to 3 nm obtained with Pt/C electron beam gun replication. Sub-populations of membrane-associated particles with ample substructure could be distinguished with Ta/W rotary-replication. For freeze-substitution the samples were after freeze-fixation kept in the substitution medium, acetone with 0.1% uranyl acetate, at -80°C for 3 days in the absence of other chemical fixatives. Specimens were then infiltrated with Lowicryl K11M for three days at -60°C and embedded in this resin for 20 days under ultraviolet polymerization, during which period the temperature was raised from -60°C to room temperature (*after* 2). The fine structural preservation is at least as good than when freeze-substitution was carried in combination with OsO_4 fixation. Lectin cytochemistry was applied on sections. Both combinations of methods, freeze-etching and Ta/W rotary-replication, and acetone freeze-substitution and Lowicryl K11M embedding of unfixed samples, can be used for lectin cytochemistry: Membranes of cellular appendices in the olfactory epithelial surface, namely those of cilia of olfactory receptor cells and of microvilli of olfactory supporting cells, contain Con A binding sites, but the density of these binding sites is higher on membranes of the latter structures than on those of the cilia. **1. Menco, B. Ph. M., Minner, E. W. & Farbman, A. I. (1988) J. Electron Microsc. Techn.: In Press; 2. Bridgman, P. C. & Phillips, G. (1987) J. Cell Biol., *105*: 224a.** *Supported by NIH Grant NS 21555, a Northwestern University's Research Committee Grant and by a gift.*

466.11

SPECIFIC FUNCTIONAL MARKERS FOR GUSTATORY NEURONS AND TASTE BUD INNERVATION IN THE RAT BASED ON LECTIN, FRAP AND CGRP. J.D. Silverman & M.Zalewski*, S. Wong*, and L. Kruger. Depts. of Anatomy and Anesthesiology, and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Primary gustatory neurons and their terminals were evaluated histochemically in geniculate ganglion (Gen g), taste buds of lingual fungiform papillae, the solitary nuclear complex (NS), and the area postrema (AP), using: a) calcitonin gene-related peptide-like immunoreactivity (CGRP-IR), marking mostly known peptidergic peripheral neurons and fibers; b) Griffonia Simplicifolia isolectin IB_4 reactivity (GSAR), specific for D-galactosyl residues and labelling largely non-peptidergic neurons and axons; c) fluoride-resistant acid phosphatase (FRAP) activity, also staining non-peptidergic neurons and co-localized with most GSAR(+) cells and central terminals.

CGRP-IR labeled rare somata in Gen g, while GSAR and FRAP stained the vast majority. Centrally, sparse CGRP-IR was found in the rostral NS and AP; GSAR and FRAP, however, robustly labeled this zone. Peripheral perigemmal CGRP-IR axons were abundant, but few intragemmal IR axons were seen. In contrast, GSAR labeled few perigemmal but numerous intragemmal axons directly beneath and between taste bud cells.

This work supports previous data showing abundant perigemmal, but sparse intragemmal peptidergic taste bud axons; and suggests GSAR to be a specific label for both intragemmal axons and FRAP(+) peripheral and central terminals. Prominent labeling of the Gen g, NS, as well as the AP, imply that GSAR may be a useful probe for examining the rat peripheral gustatory, and other visceral chemosensory neuronal systems. (Supported by a NIH grant NS-5685).

466.12

DEVELOPMENT OF OLFACTORY MARKER PROTEIN (OMP) IN THE RECEPTOR EPITHELIUM AND BRAIN IN THE MOUSE AS WELL AS ITS RELATIONSHIP TO TYROSINE HYDROXYLASE (TH) EXPRESSION. H. Baker. Dept. Neurology, Cornell Univ. Med. Coll, White Plain, NY 10605.

Until recently OMP was detected only in mature olfactory receptor neurons, but now has been localized in a number of brain regions in adult mouse. This observation prompted a study of the ontogeny of OMP in mouse CNS. A rostral-caudal gradient in OMP expression existed with cells found first in olfactory epithelium [embryonic (E) day 16], next in hypothalamus [postnatal day (P) 2] and lastly in spinal cord [P-15]. These experiments also demonstrated developmental relationships between OMP containing peripheral afferent fibers and expression of the catecholamine biosynthetic enzyme, TH, in juxtaglomerular neurons of main olfactory bulb. In E-18 through P-5 mice, OMP-containing fibers left the glomeruli, traversed the external plexiform layer and formed a dense plexus in the mitral cell layer where they contacted TH-containing neurons and processes. These data demonstrate, first that OMP in CNS, as in epithelium, exhibits developmental regulation, and second; that during development as in adults, peripheral afferents exert a regulatory influence on TH expression in dopamine neurons of the MOB. Supported by grant #NS23103.

466.13

MONOCLONAL ANTIBODY TO SUSTENTACULAR-LIKE CELLS OF THE FROG AND RAT OLFACTORY EPITHELIUM.
M.J. Crowe and S.K. Pixley* (SPON: P. Tornheim). Dept. of Anatomy and Cell Biology, University of Cincinnati, 231 Bethesda Ave., Cincinnati, OH 45267.

Few cell-type-specific antibodies exist for olfactory epithelial cells. In order to generate monoclonal antibody markers for olfactory cells that would cross-react with both rat and frog, unfixed nasal tissue homogenates from bullfrogs (Rana catesbeiana) or Sprague-Dawley rats were alternately injected intraperitoneally into a CB6F1/J mouse at two week intervals. Spleen cells from the mouse were fused with X-63-Ag8.653 myeloma cells, plated onto peritoneal exudate cell feeder plates in HAT selective medium and positive clones were chosen by immunocytochemistry on cryostat sections of bullfrog olfactory epithelium.

Supernatant from the 1D9.B9 clone demonstrated reactivity for sustentacular cells of bullfrog olfactory epithelium, using indirect immunofluorescence. Frog olfactory neurons and nerve tracts were negative. On 4% paraformaldehyde perfused, cryostat sections of rat olfactory epithelium, 1D9. supernatant showed binding to a subset of sustentacular-like cells. No staining of olfactory neurons or nerve bundles was observed. In cell cultures of adult rat nasal tissue, 1D9. immunocytochemical staining was observed on cells that were large, polygonal and rare in culture. The 1D9. antigen was internal and associated with the cell membrane. Further characterization is being done.

The monoclonal antibody, 1D9., promises to be a powerful *in vitro and in situ* for investigations of olfactory epithelium biology. (Supported by NIH PO1 NS23348-03, NSF BNS8544025 and NIH NS23523.)

466.14

DIFFERENTIATION OF OLFACTORY SENSORY NEURONS IN THE ABSENCE OF THEIR POSTSYNAPTIC TARGET.
J.E.Schwob, K.E.Szumowski*, and K.B.Brodie*. Dept. Anat. & Cell Biol., SUNY Health Science Ctr., Syracuse, NY 13210.

Olfactory bulb ablation causes olfactory sensory neuron (OSN) death followed by partial reconstitution of the neuronal population (Costanzo and Graziadei, 1983). Consequently, the morphological and biochemical differentiation of OSN's was studied after bulb ablation in adult rats with LM, EM and immunohistochemistry in an attempt to define which of the normal phenotypic properties require the influence of the olfactory bulb. There are three major findings. First, the OSN's on the ablated side are morphologically "immature", since the olfactory vesicles bear poorly developed, short cilia. Thus, differentiation of OSN's (or survival of fully differentiated neurons) is dependent on the bulb, paralleling observations during fetal development (Farbman and co-workers). Second, the uniquely "juvenile" phenotype of OSN's (vimentin expression, lack of neurofilaments (NF) and Thy-1) is independent of the bulb, as is the small subpopulation of OSN's that appear more "mature" and express NF or Thy-1 expression. Third, the expression of the cell surface, position-specific RB-8 antigen is restricted to neurons of the ventrolateral olfactory epithelium and their axons as on the control side and hence, does not require the continued presence of the postsynaptic target. Supported by NIH NS 19658 and BRSG 54026.

SPROUTING AND SPROUTING MECHANISMS II

467.1

INSULIN AND INSULINLIKE GROWTH FACTOR-I INCREASE NEUROFILAMENT mRNA LEVELS AND NEURITE FORMATION.
C. Wang*, B. Wible*, K. Angelides*, and D.N. Ishii (SPON: K.G. Beam) Physiology Dept., Colorado State Univ., Ft. Collins, CO 80523; Physiology and Molecular Biophysics Dept., Baylor College of Medicine, Houston, TX 77030.

Insulin and insulinlike growth factors (IGFs) can enhance neurite outgrowth in cultured sympathetic, sensory, and human neuroblastoma SH-SY5Y cells. The neurofilament (NF) proteins are major cytoskeletal macromolecules of axons and dendrites. To study the mechanism regulating neurite formation, a nick-translated cDNA clone containing the coding region of the human 68 kD NF protein was hybridized to Northern blots holding equivalent amounts of total or poly(A)+ RNA from cultured SH-SY5Y cells. Physiologically relevant concentrations of insulin and IGF-I substantially increased the relative abundance of NF mRNAs. The active concentrations were very similar to those that increased neurite formation. In the presence of actinomycin D, the relative degradation rate of 68 kD NF mRNAs was slower in insulin-treated than in untreated cultures. Insulin and IGFs can also stabilize and elevate tubulin, but not actin or histone, mRNAs in SH-SY5Y cells (Mill et al., Proc. Natl. Acad. Sci. USA 82: 7126, 1985). These results together suggest that neuritogenic polypeptides may coordinately stabilize and elevate transcripts coding for various cytoskeletal proteins during neurite formation. (Supported by NIH grants R01 NS24787 and NS24606).

467.2

DIFFERENCES IN THE ADHESIVE PROPERTIES OF ASTROCYTES IN THE LESIONED ADULT RAT OPTIC NERVE.
S. David and N. Giftochristos*, Neurosci. Unit, Mon. Gen. Hosp. Res. Inst., Montreal, Canada, H3G 1A4.

We have previously shown that the sprouting of injured axons in the intracranially transected adult rat optic nerve is associated with astrocyte containing, laminin$^-$ regions of the nerve near the site of lesion (J. Neurocytol. In Press). In the present study we have used an in vitro assay to examine the adhesive properties of the lesioned optic nerve.

The right optic nerve of adult Sprague Dawley rats were transected either intracranially or intraorbitally. After 5 days the animals were perfused with PBS, the lesioned optic nerves removed and 10 µm cryostat sections picked up on poly-L-lysine coated glass coverslips. PC-12 cells were seeded on to these sections ($5x10^3$ cells/well) in 24-well culture plates. After 7 days the cultures were fixed and labelled with Nuclear Yellow and anti-GFAP. Attachment of PC-12 cells was 5 to 10 fold greater near the site of the lesion as compared to regions away from the lesion. The cells were generally attached to GFAP+ areas of the lesioned optic nerve sections.

These results suggest that astrocytes near the site of a lesion may differ in their adhesive properties, from those located away from the lesion.

467.3

SPROUTING OF CGRP NERVE FIBERS IN RESPONSE TO DENTAL INJURIES.
P.E. Taylor* and M.R. Byers (SPON: B.R. Fink). Depts. of Endodontics; Anesthesiology; and Biol. Structure; Univ. of Washington, Seattle, WA 98195.

Calcitonin gene-related peptide (CGRP) immunocytochemistry has been used to demonstrate a large population of unmyelinated sensory nerves. Recently these nerves and the various neuropeptides that they contain have been implicated as mediators of inflammation and healing responses. The purpose of this study was to determine the response of these nerves to dental injuries.

Right first molars of 40 adult male Sprague Dawley rats received one of 3 degrees of injury: mild, outer dentin cavity; moderate, inner dentin cavity; severe, pulpal exposure. After 1 to 35 days the rats were sacrificed. The molar teeth were fixed, decalcified, sectioned and stained using standard immunocytochemistry for CGRP, then prepared for LM or EM evaluation. Control teeth showed a network of CGRP immunoreactive (IR) nerve fibers consistent with known dental sensory fiber distribution. The experimental teeth showed CGRP-IR fibers sprouting either into or around the affected tissue depending on severity of the injury. EM showed that the sprouting zone contained both CGRP-IR and unstained nerve fibers. In the milder injuries these responses were greatest 4 days post injury and recovered by 10-21 days. Preliminary studies of denervated teeth suggest that pulpal healing after severe injury is compromised in the absence of sensory nerve fibers.

This study was supported by NIH grant #DE05159.

467.4

INTERACTIONS OF NERVES AND PULP CELLS IN NORMAL AND INJURED TEETH ANALYSED BY LM AND EM IMMUNOCYTOCHEMISTRY FOR NGF-RECEPTOR.
M.R.Byers, M.A.Bothwell, K.B.Mecifi*, and G.C.Schatteman*. Anesthesiology; Physiology and Biophysics; Biol. Structure; Univ. of Washington, Seattle, WA 98195.

NGF cell surface receptor (NGFR) immunocytochemistry is useful for detecting cells or cell interactions which may be dependant on NGF. We used a monoclonal antibody to rat NGFR (Chandler et al, J.Biol.Chem 259:6882, 1984) to study its expression in axons, free nerve endings (FNE), Schwann cells (SC), odontoblasts (OD) and fibroblasts (FB) in normal rat molars or ones injured by drilling superficial dentin 4d prior to fixation. LM and EM preembedment methods were used with the avidin-biotin-peroxidase-DAB reaction. Normal Molars: NGFR-immunoreactivity (IR) was found on cell membranes of unmyel.axons and their SC; on FB in peripheral coronal pulp; and on many FNE. NGFR-IR was absent from myel. axons, some of their unmyel. terminal branches, and some FNE. OD were not stained except at contacts with labeled FB, axons or FNE. Injured 4d: FB stain was gone. Unmyel. axons and their SC were more numerous and had increased NGFR-IR. OD were proliferating and making reparative dentin; they had no NGFR-IR except at contacts with stained nerve fibers. Our results suggest that there may be NGF-dependant interactions in teeth that involve unmyel. axons, SC, some FNE, FB, and possibly OD at sites of contact with FB and nerves. NGF-immunoreactivity is also being studied in these teeth. (Supported by NIH grants #DE05159 and NS23343.)

467.5

LAMININ PROMOTES MOTOR NERVE SPROUTING IN VIVO. W.C. Yee*, A. Postronk and A.F. Hahn*(SPON:F.LaBella). Univ. of Man., Winnipeg, Canada; Johns Hopkins Sch. of Med., Baltimore,MD, 21205; Univ. of Western Ontario, London, Canada.

Extracellular matrix proteins have been reported to promote neurite outgrowth in vitro. We studied the effect of administered laminin and fibronectin on an in vivo model of motor nerve sprouting. Nerve terminal sprouting was induced at neuromuscular junctions(NMJs) of soleus muscles of adult Swiss mice by direct injection of botulinum toxin.Following botulinum treatment, daily injections of laminin or fibronection, 1mg/ml in Ringer's solution, were given directly into the muscles. Bovine serum albumin(BSA) in Ringer's or Ringer's solution alone was injected into control muscles. Muscles were removed on the 7th day. Thick longitudinal cryostat sections of muscles were stained with a combined silver-cholinesterase technique to demonstrate nerve terminals and sprouts. Tissue fixed in 3% gluteraldehyde was processed for electronmicroscopy(EM). In laminin injected muscles stained with the silver-cholinesterase technique, with botulinum induced sprouting, nerve terminals showed unusual nodular or tufted expansions. EM demonstrated multiple nerve sprouts and Schwann cell processes crowded into primary postsynaptic folds, accounting for the expansions on light microscopy. Nerve terminal expansions and multiple sprout crowding were not seen in muscles injected with fibronectin, BSA or Ringer's. Our findings suggest that administration of laminin promotes the proliferation of sprouts during motor nerve sprouting in vivo.

467.6

5-HT IMMUNOCYTOCHEMISTRY AND FRAP HISTOCHEMISTRY DEMONSTRATE NEURONAL PLASTICITY OF TWO CONVERGENT SYSTEMS IN THE ADULT RAT SPINAL CORD. D.C. Polistina and M.E. Goldberger. Medical College of Pennsylvania, Phila., PA 19129.

In a normal adult rat spinal cord, the superficial dorsal horn receives 5-HT projections from the midline raphe' nucleus and FRAP projections from the dorsal root ganglion and each of these converging projections are symmetrical from side to side. After unilateral partial deafferentation (sparing L5) the chronic (>30 days) spared root side shows an increase in 5-HT immunoreactivity in the superficial dorsal horn in segments rostral and caudal but not at the spared root's segment of entry (L5). In control bilateral spared root animals the distribution of the L5 dorsal root is symmetrical in lamina II of the dorsal horn as demonstrated by FRAP histochemistry. In experimental bilateral spared root animals, the chronic (>30 days) spared root side shows an increase in FRAP labeling within its normal distribution compared to the acute (4 days) spared root side. These results are similar to the results obtained by McNiel and Hulsebosch (1987) except that the spread of FRAP beyond the spared root's normal distribution was not seen in the present study. These data suggest that a projection may increase in response to partial deafferentation. In the case of converging projections the pathway more closely related to the one removed, in this case the spared dorsal root, prevents the increase of the 5-HT projection within the spared root's normal projection area.

Supported by NIH grants NS24707,NS16629,& NSF grant NS8605441.

467.7

THE RESPONSE OF SPARED DORSAL ROOT AFFERENT COLLATERALS FOLLOWING DORSAL FUNICULUS LESIONS IN JUVENILE XENOPUS LAEVIS. H.L. Campbell, M.S. Beattie, and J.C. Bresnahan. Dept. of Anat., Div. Neurosurg., and Neurosci. Program, Ohio State Univ. Coll. Med., Columbus, OH 43210.

The response to axotomy of dorsal root axons travelling in the dosal columns [DC] was studied in xenopus juveniles to determine the effects of 'pruning' rostral DC collaterals on segmental innervation patterns. Unilateral DC lesions just rostral to the lumbar enlargement were made in juvenile frogs. 3 days to 13 weeks later, axons of the tenth spinal nerve were filled with HRP. Axons extending into the lesion site appeared disorganized and often exhibited large swellings. Axons were not observed to extend through or around the lesion sites. Caudal to the lesion site, in the region of the tenth root entry zone, several phenomenon were noted: the area and density of the dorsal and ventral terminal fields [DTF and VTF respectively] increased significantly; the number of axons crossing the midline at the tenth segment and the extent of their contralateral arborizations increased and the diameter of axonal swellings located in the VTF appeared to increase. (Supported by NS-10165)

467.8

EVIDENCE FOR SPROUTING OF DORSAL ROOT TERMINAL FIELDS AFTER SPINAL CORD HEMISECTION IN JUVENILE **XENOPUS** FROGS. D. Norris*, M.S. Beattie, and J.C. Bresnahan. Depts. of Anatomy and Surgery, Ohio State Univ., Columbus, OH., 43210.

Complete spinal cord hemisections result in the removal of descending inputs to the spinal gray, and also axotomize the ascending collaterals of dorsal root axons innervating the cord caudal to the lesion.

Hemisections were made just rostral to the lumbar enlargement in juvenile Xenopus laevis frogs anesthetized with MS 222. Five weeks later, the tenth spinal nerves were labelled with HRP bilaterally to visualize the density and distribution patterns of dorsal root afferents caudal to the lesion. Tissue was processed using the CoCl-enhanced DAB procedure.

Six of seven cases were judged blind by two independent observers to have a greater innervation density and terminal field size on the side of the lesion for both dorsal and ventral terminal fields. Volumetric image/density analysis is underway to substantiate these observations. Hemisection appears to induce sprouting of dorsal root afferents in Xenopus. (Supported by NS-10165).

467.9

SPROUTING IN THE ELECTROSENSORY LATERAL LINE LOBE OF TELEOSTS FOLLOWING RICIN ABLATION. M.J. Lannoo* and L. Maler (SPON: W. Hendelman). Anat. Dept., University of Ottawa, Ottawa, Ontario, K1H 8M5.

Sprouting of primary electrosensory afferents occurs in the electrosensory lateral line lobe (ELL) of the teleost Apteronotus following ablation of peripheral nerve branches with Ricinus communis. We injected 2 ul of 2 ug/ml ricin diluted with fast green (Wiley and Oeltmann, '86) into a single nerve branch. Anterior lateral line ganglion cells became pyknotic 3-5 days following ricin injection. At two week intervals up to four months we double-labelled two of the remaining three nerve branches using the HRP-Cobalt technique (Ebbesson and Bazer, '87) and examined preparations for sprouting. The ELL is composed of four, somatotopic maps. Following supraorbital nerve branch ablation, infraorbital fibers sprouted from terminal arbors into this denervated area by two weeks in the medial and centromedial maps of the ELL. By four weeks sprouting from the infraorbital nerve had begun in all four maps; by six weeks sprouting was robust. Because adjacent segments are mirror-image oriented, sprouting fibers in adjacent segments coursed in opposite directions. Sprouting also occurred in ventral fibers to deeper parts of the ELL; these sprouting fibers coursed dorsally and probably originated from nodes of Ranvier. At four weeks, sprouting fibers from the trunk nerve also invaded the denervated supraorbital regions. Competition probably occurs between the infraorbital and trunk nerves for synaptic space.

467.10

MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHANGES ELICITED IN MOUSE MOTOR NERVE TERMINALS BY IN VIVO INJECTION OF BOTULIN A TOXIN. D. Angaut-Petit* and J. Molgo* (SPON: Y. Padel). Lab. Neurobiol. Cell. and Molec. CNRS, 91190 Gif sur Yvette, France.

A sublethal dose of Botulinum A neurotoxin administered locally, *in vivo*, on the levator auris longus muscle of the mouse causes paralysis of the muscle and triggers the growth of motor nerve terminals. Between 12 and 20 days after the injection, these terminal sprouts stained by silver impregnation or by fluorescent mitochondrial dyes appeared as thin and long (100μm) filaments oriented parallel to the muscle fibres. An *in vitro* study of ionic permeabilities of the membrane of preexisting motor endings and newly formed sprouts was performed with the aid of specific channel blockers. The results revealed that: 1) the sprout growth is accompanied by the appearance of net inward current along the preexisting nerve terminal membrane, 2) the newly formed membrane also conducts impulses. Labelling of muscle acethylcholine receptors with rhodamine-conjugated α-bungarotoxin suggests their presence along the terminal outgrowth.

467.11

AGE-RELATED CHANGES IN ULTRATERMINAL SPROUTING WITHIN* DIFFERENT MUSCLES. J.L. Rosenheimer, L.A. Trotter and D.O. Smith. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

End plates from extensor digitorum longus (EDL), soleus and diaphragm muscles of 10- and 25-mos rats were examined for the effect of aging on ultraterminal sprouting, a form of terminal sprouting usually associated with muscle denervation. There was a significant age-related increase in the fraction of end plates exhibiting ultraterminal sprouting in the EDL muscle; the number increased from 5.5% to 23.0%. There were comparatively fewer end plates with ultraterminal sprouts (< 3.0%) in the soleus and diaphragm muscles of 10-mos rats, and this number did not change significantly with age. Sprout length did not exhibit an age-related change in any of the muscles, although sprouts were consistently longer in the EDL. End plates from muscles of 25-mos animals that had been chronically stressed or exercised were also analyzed. These extrinsic factors did not alter the number of end plates exhibiting ultraterminal sprouting in any of the muscles. It is concluded that in certain muscles, aging may be accompanied by changes in the relationship between the nerve and muscle that may induce the expression of denervation-like characteristics which are not influenced by extrinsic factors previously shown to affect terminal number. Supported by NIH grants AG01572 and AG05340.

DEVELOPMENTAL DISORDERS

468.1

PRAGMATIC LANGUAGE SKILLS IN CHILDREN WITH SCHIZOPHRENIA. J.G. FOY* and R. CAPLAN. 48-241 Neuropsychiatric Ins., UCLA, 760 Westwood Plaza, Los Angeles, CA 90024

Pragmatic language skills have been found to be impaired in adult schizophrenics (e.g., Rochester & Martin,1979) The purpose of this study was to investigate the pragmatic skills of children with schizophrenia and to elucidate the nature of these deficits.

15 schizophrenic children aged 7 -12.5 years were matched by sex, MA and SES to normal children. The schizophrenic children were recruited from UCLA's Neuropsychiatric Institute Inpatient and Outpatient Children's Services and from 2 L.A. schools for the emotionally disturbed. They were independently diagnosed with the Interview for Childhood Disorders and Schizophrenia (ICDS) by the Diagnostic Unit of the Childhood Psychosis Clinical Research Center. Language samples were obtained from videotapes of the Kiddie Formal Thought Disorder Story Game (SG) developed by Caplan (1988). The transcribed videotapes were rated with the Pragmatic Skills Scale (PSS) by 2 trained, blind raters. This instrument was adapted from Halliday and Hasan (1976) and operationalized for use with children.

Preliminary results indicate that the schizophrenic children differ from normal children in their use of endophora and unknown ties. The relationship of these behaviors to age and diagnosis will be presented in detail.

468.2

MONOAMINE OXIDASE ABSENT IN NORRIE DISEASE PATIENTS WITH DELETION IN CHROMOSOMAL REGION Xp11.3. M.Utterback,K.Sims*,A.dela Chapelle*, R.Norio*,E.Sankila*,Y-P.Hsu*,J.Powell*,J.Gusella X.Breakefield. Neurogenetics Div.,Shriver Ctr., Waltham, MA; Neurogenetics Lab, Mass.Gen.Hosp.; Med.Gen.Div.,Univ.Helesinki; Endocrin.Res.Unit, Harrow,England; Clin.Sci.Lab, NIMH.

Moroamine oxidase (MAO), in its two forms MAO-A and MAO-B, is the enzyme primarily responsible for degradation of amine neurotransmitters. A complementary DNA (cDNA) clone for human MAO-A has been used to establish the deletion of its corresponding gene in two male cousins with Norrie disease. No MAO-A activity was detected in their fibroblasts.MAO-B activity in platelets and fibroblasts from these patients was also non-detectable. Moreover, major catecholamine metabolites, including vanillylmandelic acid (VMA), homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG), were reduced substantially in their urine. These findings indicate that gene(s) necessary for MAO-A and MAO-B activities are deleted in these patients and that the gene loci are near each other in Xp11.3. This is the first report of absent MAO activity in humans; at least some of the clinical features of these patients may reflect this deficiency.

468.3

CORRELATION OF MORPHOLOGICAL, BIOCHEMICAL AND CBF CHANGES IN EXPERIMENTAL INFANTILE HYDROCEPHALUS. U.A. Vasthare*, R.F. Tuma*, I.A. Van Orden*, L.C. Wright*, S.D. Katz*, T.J. Lovely*, R.H. Rosenwasser* and J.P. McAllister. (SPON:J.A. Kenning). Depts. of Anatomy, Physiology and Neurosurgery, Temple U. Sch. Med., Philadelphia, PA 19140.

Our previous studies have shown that pyramidal cells degenerate and monoamine levels are reduced in cerebral cortex of hydrocephalic kittens. The present study sought to determine the relationship between these morphologic and biochemical changes and cerebral blood flow (CBF). Hydrocephalus was induced in 4-10 day old kittens by intracisternal injections of 25% kaolin and verified with ultrasound; control animals received similar injections of sterile saline. The isotope labelled microsphere method was used to measure CBF 15-20 days post-injection. Significant ($p<0.05$) decreases in CBF were detected Areas 4 (55%), 22 (56%) and 17 (58%) of cortex, as well as thalamus (46%), midbrain and pons (55%), cerebellum (50%) and caudate nucleus (61%). A Cushing response was evidenced by decreases in heart rate (22%) and cardiac output (70%), and a 221% increase in total peripheral resistance. The changes in cortical CBF did not follow the rostrocaudal gradient of neuronal degeneration and monoamine changes. Thus, these data suggest that reductions in cortical CBF may not be the only factor mediating the neurologic deficits or the neuronal deterioration that accompany hydrocephalus. Supported by NIH Grant SO RR05417 to JPM.

468.4

INTERREGIONAL CORRELATIONS OF GLUCOSE UTILIZATION AMONG BRAIN REGIONS IN YOUNG DOWN SYNDROME (DS) ADULTS. B. Horwitz, M. Schapiro*, C. Grady, and S. I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892.

Correlations between regional cerebral metabolic rates for glucose (rCMRglc), determined by positron emission tomography using [^{18}F]fluorodeoxyglucose provide a measure of the functional associations between pairs of brain regions (Horwitz et al., J. Cereb. Blood Flow Metabol., 4, 484-499, 1984). We applied this method to healthy, trisomy 21 DS adults (23-33 yrs) and to 24 age-matched healthy controls (20-35 yrs).

Correlations were obtained between ratios of resting rCMRglc to global brain metabolism (Q-values). Most Q-values had group means that did not differ significantly ($p > 0.05$) between DS subjects and controls. However, many correlations between regions within and between the frontal and parietal lobes had significantly ($p < 0.01$) lower values in the DS group than in controls, with some being large and negative in the DS group that were large and positive in the controls. One region so affected was the left inferior frontal gyrus that includes Broca's area.

The decreased values of correlations associated with Broca's region are consistent with the relatively greater language impairment seen in subjects with DS. The reduced values of frontal-parietal correlations in the DS group are similar to that found in adult autistic patients (Horwitz et al., Arch. Neurol., in press), and can be interpreted as indicating a disruption of neural systems associated with directed attention.

468.5

MICROGLIA RESPONSES DURING DEVELOPMENT IN DOWN'S SYNDROME. L.C. Stanley*, L.J. Perrot, and W.S.T. Griffin (SPON: D.L. Davies). Depts. of Anat. and Pediat., Univ. Arkansas Med. Sci., Little Rock, AR 72205

Recently, we noted a profound astrogliosis characterized by elevated S100 immunoreactivity present in Down's syndrome (DS) as early as 2 days postnatal. As microglia produce factors, including Interleukin 1 (Il-1), that stimulate astrocytes in culture, we postulated that during development microglia become reactive and produce Il-1 which may contribute to the reactive astrogliosis we show in DS. Rabbit-anti-human Il-1 (1:500) was immunoreacted with sections of formalin-fixed, paraffin-embedded temporal lobe from cases of DS (ages 18-19 weeks gestational, 2 days and 3.5 months postnatal) and age-matched controls (AMC). Diaminobenzidine was used to stain immunoreactive products. There were greater numbers of Il-1 immunoreactive glia present in DS compared to AMC, and Il-1 immunoreactivity was dramatically elevated in what were morphologically identified as reactive microglia in the DS tissue. Because of the ability of Il-1 to initiate the release of corticotropin releasing factor, adrenocorticotropic hormone, and hydrocortisone and thereby cause immunosuppression, the elevation of glia-derived Il-1 may also contribute to the systemic immunodepression syndrome that accompanies DS.

468.6

ASTROCYTIC RESPONSE IN DEVELOPMENT DURING DOWN'S SYNDROME. W.S.T. Griffin, L.J. Perrot, and L.C. Stanley*. Depts. of Pediat. and Anat., Univ. Arkansas Med. Sci., Little Rock, AR 72205.

Neuropathology similar to that in Alzheimer's disease is present in Down's syndrome (DS) after age thirty (Wisniewski, K.E., Ann. Neurol., 17:278, 1985). Recently, the S100 gene was localized to chromosome 21 (Allore, R., Science, 239:1311, 1988). Since S100 is induced in reactive astrocytes, the age-related presence of reactive astrocytes in DS may be related to the gene duplication in trisomy 21. We postulated that during development, astrocytes become reactive and produce elevated levels of S100 in DS. Rabbit-anti-cow S100, diluted 1:300, was immunoreacted with sections of formalin-fixed, paraffin-embedded temporal lobe from cases of DS (ages 18-19 wk. gestational; 2 d. and 3.5 mo. postnatal) and age-matched controls (AMC). The specific astrocyte marker, glial fibrillary acidic protein (GFAP), diluted 1:500, was similarly immunoreacted. Diaminobenzidine was used to stain immunoreactive products (IR). We found greater numbers of GFAP-IR and S100-IR reactive astrocytes in DS compared to AMC, and the immunoreactivity per cell was dramatically elevated. Besides its relationship to astrocyte reactivity suggested here, overexpression of the S100 gene in DS may contribute to microtubule assembly dysfunction since S100 inhibits ATPase activity needed for microtubule assembly [Fujii, T., Chem. Pharm. Bull., 35(10)4324, 1987].

468.7

EXPRESSION OF (1) A CELL ADHESION MOLECULE (N-CAM) AND (2) A NEURITE PROMOTING MOLECULE (LAMININ) IN POST-MORTEM BRAIN TISSUE FROM ALZHIEMER'S DISEASE AND DOWN SYNDROME PATIENTS. S-J. Richards & P. Liesi*, Dept, of Biochemistry & Molecular Genetics, St. Mary's Hospital Medical School, London W2 1PG, UK and *Recombinant DNA Laboratory, University of Helsinki, Valimotie 7, Helsinki.

Down syndrome is a genetic disorder in which affected persons have a karyotype of either trisomy 21 or a translocation of the long arm of chromosome 21, in particular the region 21q22.1-21q22.2. It is the altered expression of the genes within this 'pathological' region of chromosome 21 that is thought to account for the increased incidence of Alzheimer's disease observed.

Previous neurological studies have reported that the increase in genetic material associated with the extra copy or portion of chromosome 21 causes neuronal cytoskeletal abnormalities and which may be causally related to the development of Alzheimer's disease.

We have speculated that the neurological abnormalities may be related to the over-expression of a gene(s) involved in embryogenesis, and further suggest this gene(s) could be a growth factor, cell adhesion molecule or a molecule involved in neuronal migration and axonal guidance.

To date we have examined levels of expression of N-cam and laminin in post-mortem brain material using Northern blot analysis. We are unable to report a significant variation in expression of either of these two genes within control and experimental brain tissues.(SPON: R.C.A. Pearson)

468.8

VOLTAGE DEPENDENCY ON ELECTRICAL MEMBRANE PROPERTIES IN TRISOMY 16 AND NORMAL MOUSE DORSAL ROOT GANGLIA NEURONS IN CULTURE. **J. Hidalgo,* B. Ault* and S. I. Rapoport (Spon. Anne E. Schaffner)**
NIH, NIA Lab. of Neurosciences. Bethesda MD 20892

Our laboratory has reported that trisomy 16 dorsal root ganglia (DRG) embryonic mouse neurons in culture present several electrical properties that differ from paired litter controls. Since some of these properties have a strong voltage dependency, experiments were performed to establish to what extent differences in the membrane potential could account for them.

Primary cultures of DRG neurons were kept for 2 to 4 weeks. The whole cell variation of patch clamp recording was used in current clamp mode. Standard solutions of K^+ and Na^+ for internal and external mediums were used. Current was applied to hold the membrane potential at levels between -45 and -65 mV. Suprathreshold current pulses of 10 msec were used to elicit action potentials.

The resulting data was digitized, analyzed and tabulated. In trisomy 16 neurons, the time for peak during hyperpolarizing after potential (HAP) is more sensitive to voltage and the rate of repolarization is less sensitive to voltage than in control neurons. The calcium and potassium conductances that could be underlying these differences are being studied at the single channel level.

468.9

ULTRASTRUCTURAL CHARACTERISTICS OF PERIPHERAL VISUAL AND AUDITORY SYSTEMS IN CAPRINE β-MANNOSIDOSIS. **J. A. Render*, K. L. Lovell and M. Z. Jones. Dept. Pathology, Mich. State Univ., East Lansing, MI 48824.**

Caprine β-mannosidosis, an autosomal recessive defect of glycoprotein catabolism associated with a deficiency of tissue and plasma lysosomal β-mannosidase, is expressed at birth by severe neurological deficits, including deafness. Affected kids have normal light reflexes. In the present study, ultrastructural abnormalities of the peripheral visual and auditory systems in affected goats were defined in order to further investigate the expression of the disease in peripheral sensory organs and the correlation between morphological changes and sensory function. With respect to the visual system, many types of ocular cells contained intracytoplasmic vacuoles; vacuolated retinal cells included photoreceptor cells, cells within the inner nuclear layer, and ganglion cells. Ultrastructurally these vacuoles were membrane bound, occasionally coalescing and principally electronlucent. In the optic nerve, vacuolated glial cells and ovoidal axonal spheroids composed mainly of dense bodies were present. These lesions and severe myelin deficits in optic nerve apparently do not interfere substantially with visual function. Otic morphologic abnormalities involved the external, middle and inner ears. Alterations involving the cochlea included cytoplasmic vacuolation of most cells. Through continued research on β-mannosidosis, we hope to understand the correlation between morphological changes and function of the special senses in storage diseases. Supported by NS16886 to M.Z.J.

468.10

A PERFORMANCE DEFICIT IN THE ADULT OFFSPRING OF C57 MICE EXPOSED TO ETHANOL DURING EARLY GESTATION. R. Dumas* and E. A. Sersen* (SPON: G.Y. Wen). NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Although the deleterious effects of a brief exposure to a large dose of alcohol on the morphological development of the mouse fetus have been delineated, the subsequent functional consequences of such an exposure are not known. Previous work in our laboratory indicated deficits in climbing behavior of mice with normal external morphology born to C57Bl/6J dams gavaged with 5.8 g/kg of ethanol on E9. Recently, a larger sample of 3 groups of pregnant mice (E, ethanol; D, isocaloric dextrose; C, untreated) were allowed to come to term. Neonatal mortality and hydrocephaly, developing within the first 3 weeks, reduced the E offspring by 30%. The remaining E mice and their controls were tested on a wire bar vertical plane at 28 d and then weekly for a total of 13 weeks. The E offspring displayed essentially normal activity and motor coordination, but their movements were slower than those of the controls in traversing the apparatus. They showed a marked and significant ($p<.005$) failure to negotiate a small drop at the bottom of the plane to return to the home cage. This finding suggests a persisting functional impairment in adult normal-appearing mice who had been briefly exposed to ethanol during a critical stage of embryonic development.

468.11

THE RAT WITH METHYLAZOXYMETHANOL-INDUCED MICRENCEPHALY - A MODEL FOR DEVELOPMENTAL ABNORMALITIES OF THE VISUAL SYSTEM. A. Rabe, M. H. Lee and P. Wang*. NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

The micrencephalic rat with its hypoplasia of the forebrain is a useful model for the study of developmental brain defects. The two most consistently seen behavioral changes of this rat are maze learning deficits and hyperactivity. Both are unspecific since each may arise from morphological and neurochemical alterations in several different brain regions. The markedly reduced occipital region, as well as neuron loss throughout the subcortical visual system of the micrencephalic rat (Ashwell, 1987), suggest that modality-specific (visual) deficits should also exist. Indeed, Pereira et al. (1985) showed micrencephalic Wistar rats to be markedly impaired in visual pattern, but not brightness, discrimination. We have now replicated their results with Long-Evans rats. Mature rats learned to discriminate between two visual stimuli to escape a mild footshock. A black and white discrimination was given to 7 micrencephalic (M) and 8 normal (C) rats, and a discrimination of horizontal vs vertical 6 mm black and white stripes was administered to other groups of 15 M and 14 C rats. The M rats were impaired in learning the pattern, but not brightness, discrimination. The scores for pattern discrimination M vs C, were: total correct trials in 10 d, 157$\pm$ 21 vs 174 $\pm$ 10, $p < .011$; total errors 59 $\pm$ 26 vs 31 $\pm$ 12, $p < .001$.

468.12

OVERPRODUCTION OF PROTEASE NEXIN I (PNI) IN RAT BRAIN TUMORS IN VIVO. J.S. Rao*, J.B. Baker*[1], and B.W. Festoff (SPON: L.T. GIRON). Neurobiol. Res. Lab (151), V.A. Med. Ctr., Kansas City, MO 64128; [1]Dept. of Biochem., Univ. of Kansas, Lawrence, KS 66045.

PNI is identical to glial derived nexin (GDN), a neurite outgrowth promotor found in rat and human glial cells. The 9L rat brain tumor is a gliosarcoma and a model of human brain tumors. Our previous results showed that these cells secrete both PNI and PAI-1 (endothelial cell-type PA inhibitor). We studied these inhibitors in normal brain and 9L tumor in vivo. A PNI-type inhibitor formed a 92 Kd and 78 Kd complex with ^{125}I-urokinase and ^{125}I-thrombin, respectively, in the absence of SDS. In the 9L tumor, the PNI complexes were increased threefold when compared to normal brain. In addition, in contrast to studies with 9L in vitro, in the presence of SDS we found another complex at 88 Kd with ^{125}I-thrombin, but no complex with ^{125}I-urokinase. Brain tumor pathogenesis may involve increased gene expression with protease nexin I and decreased PAI-1. Such studies are in progress.
Supported by the ALS Assoc. NSF, Speas Found., and the Medical Research Service of the VA.

468.13

FUNDAMENTAL MECHANISMS OF TUMORIGENESIS IN THE HUMAN NERVOUS SYSTEM. B.R.Seizinger*, G.A.Rouleau*, G.E.Farmer*, A.H.Lane*, R.L.Martuza* and J.F.Gusella*. (SPON: L.Jacoby). Depts.of Neurology and Neurosurgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

One way of gaining insights into the normal function of the human nervous system is to study its dysfunction in specific disorders. Neurofibromatosis (NF), one of the most common inherited disorders affecting the human nervous system, is typically associated with Schwann cell-derived tumors which can lead to serious neurological complications and death. Two genetically and clinically distinct forms of NF have been described, NF1 and NF2. Using a molecular genetic approach, we have provided conclusive evidence that the defective genes causing NF1 and NF2 map to chromosome 17 and chromosome 22, respectively. This provides the basis for the isolation and characterization of the NF genes, which might eventually lead to the development of effective therapies for these serious neurological disorders. We have shown that different tumor types in NF2 are specifically associated with deletions on chromosome 22 suggesting a common pathogenetic mechanism for meningiomas, acoustic neuromas, and possibly certain types of astrocytic tumors. The same gene defect reponsible for the inherited tumors in NF2 may also cause their sporadic counterparts which constitute a considerable portion of neoplasias in the human brain. The NF2 gene seems to belongs to a class of cancer-related genes, so called tumor suppressor genes, whose loss, deletion or inactivation leads to tumor formation.
We have recently provided strong evidence that a similiar mechanism of tumorigenesis, loss of a tumor suppressor gene on the short arm of chromosome 3, underlies VHL. VHL is a devastating cancer syndrome, associated with endothelial-derived tumors in the brain and spinal cord, phaeochromocytomas, and renal cell carcinoma. The isolation and characterization of these tumor suppressor genes may not only provide insights into one of the most fundamental mechanisms of cancer development in humans, but could also elucidate key mechanisms regulating the normal development and differentiation of the human nervous system.

468.14

HYPOXIA DISRUPTS NEURONAL MIGRATION IN THE RAT. J.H. Jennings*, A.W. Deckel, and M.C. Yu. (SPON: J.A. Liederman). Depts. of Psychiatry and Anatomy, Univ. of Med. and Dent., N.J. Med. Sch., 185 S. Orange Ave, Newark, N.J., 07103.

Previous work indirectly suggested that the hypermyelination found in the striatum and thalamus in individuals with cerebral palsy was secondary to an arrest of neurons migrating from the ganglionic eminence to late forming telencephalic structures, with subsequent myelination of these neurons as they differentiated (Deckel and Robinson, Exp. Neur., 91, 212-218, 1986). This experiment directly tested that hypothesis by exposing pregnant rat dams at 14, 16, or 19 days post conception to 48 hours of a 10% oxygen/90% nitrogen environment. Controls were left at room air. Animals were given an injection of tritiated thymidine (5 uCi/g; specific activity= 6.7 Ci/mMol) immediately before placement into the hypoxic environment; rat pups were sacrificed at 21 days post birth, perfused, and examined by autoradiography and Nissl histology. Cell counts indicated increase numbers of labeled neurons in the striatum and layer III of the neocortex of hypoxic animals, and decreased numbers of cells in neocortical layer VI. The effect was greatest for the animals of gestational ages E14-E16. In addition, a hypermyelination was found within the striatum of the hypoxic animals. These results indicate that perinatal rodents exposed to a hypoxic environment (1) have a disruption of neuronal migration in the striatum and neocortex, and (2) evidence a status marmoratus-like effect within the striatum. Implications of these findings for cerebral palsy are discussed.

468.15

QUANTITATIVE MORPHOLOGIC ANALYSIS OF THE EFFECT OF MILD PERINATAL ASPHYXIA ON RAT STRIATAL CHOLINERGIC NEURONS. RE Burke, RI Stark. Columbia Univ. Coll. P&S, NYC 10032.

Little is known of the effect of perinatal asphyxia on the neurochemical anatomy of the brain. Neurochemical studies show that striatal cholinergic (ACh) markers are selectively diminished in a rodent model (Johnston, 1983), but morphologic studies show preservation of striatal ACh neurons (Johnston & Hudson, 1987). We examined the effect of mild injury on the total number of these neurons, determined by counting in 8 serial planes through the striatum. We used a model of unilateral asphyxia (ligation of one carotid artery; exposure to 8% O_2). ACh neurons were stained by the immunoperoxidase method using a monoclonal antibody to CAT (B-M). Brains were fixed by immersion, because perfusion (in the presence of a ligation) led to asymmetries of staining, visible in control nuclei and ligation-alone controls. At 3 weeks, normal controls fixed by immersion were symmetrical in total striatal ACh neurons (R=5393+266 (SEM); L=5388+280; N=8) (Abercrombie corrected). Rats with unilateral (left) asphyxia unexpectedly showed a trend for an increased number of ACh neurons on the affected side (R=4371+347; L=4659+339; N=6; p=0.1). An increase was also seen in 8 week rats (R=4036+222; L=4635+193; N=7; p=.01). The increase on the asphyxiated side was uniform rostro-caudally. Striatal ACh neurons are not only resistant to asphyxial injury, but possibly increased in number by an as yet undefined mechanism.
NINCDS TIDA #1K07NS00746 Dystonia Medical Research Foundation.

468.16

HIPPOCAMPAL ANOMALIES IN THE NEW ZEALAND BLACK MOUSE: A GOLGI STUDY. A.M. Galaburda, W.E. Kaufmann and N. Jaskowiak*. Dyslexia Neuroanatomical Laboratory, Harvard Medical School and Beth Israel Hospital, Boston, MA 02215.

It is reported that NZB mice show hippocampal anomalies by the Golgi-Stensaas method (Nowakowski, R.S., *Soc. Neurosci. Abs.* 13:1117, 1987). They consist of ectopic neurons, which show abnormal dendritic arborization, in the molecular layer and in the limit between the molecular and the granule cell layer. We examined by the Nissl and Golgi-Cox-Sholl methods the hippocampal formations of 14 NZB and 6 DBA mice, and describe here neurons from CA1 and CA2 of Ammon's Horn.

No pyramidal cell layer abnormalities were noted in the Nissl-stained sections. The distribution of neurons stained by the Golgi method was more regular in the DBA than in the NZB. The apical dendrites in the NZB, but not in the DBA, were straighter and lacked the tortuosity characteristic of this region (Banker, G.A. and Cowan, W.M., *J. Comp. Neurol.*, 187:469, 1979). There was more variability in the degree of basal dendritic arborization in the NZB than in the DBA, and the general pattern of branching in the former tended to adopt the axial, rather than the more typical laminar, distribution. Some of the NZB neurons exhibited basal dendrites directed toward the *stratum oriens*. Some of the pyramidal cell changes are reminiscent of earlier hippocampal developmental stages and others suggest developmental deviance. Quantitative analysis is currently in progress and will be discussed.

(Supported by NIH-NICHD 20806 and a grant from the Carl J. Herzog Foundation).

468.17

NEOCORTICAL ANOMALIES IN THE NEW ZEALAND BLACK MOUSE: A GOLGI STUDY. W.E. Kaufmann, N. Jaskowiak* and A.M. Galaburda. Dyslexia Neuroanatomical Laboratory, Harvard Medical School and Beth Israel Hospital, Boston, MA 02215.

Some NZB mice show neocortical molecular layer ectopias and laminar dysplasias (Sherman, G.F. et al., *PNAS* (USA), 82: 8072, 1985). We compared over 500 cells from 3 NZB mice with 4 anomalies (NZBw), 8 without anomalies (NZBw/o), and 8 DBA controls for Golgi characteristics (Golgi-Stensaas). We made planimetric analyses of cell populations in the superficial portion of layer ii (iis), the deep portion of layer ii (iid), and pyramidal neurons in layers iii, v, and vi. We quantitated basal dendritic length (*bdl*), total number of basal dendritic branches (*btn*) and of 1st (*b1*), 2nd (*b2*), and 3rd (*b3*) order branches, number of terminal basal dendritic branches (*tbdb*), apical dendritic length (*adl*), cell body surface area, average length per basal dendritic branch (*bdl/btn*), and ratio of apical to basal dendritic development (*adl/bdl*).

NZBw/o showed greater *bdl, btn, b2, b3* than DBA in all layers except vi, greater *adl* in iii and v, and larger *bdl/btn* in iis and iii. NZBw had greater *tbdb* than DBA in iii. Also, DBA showed significant interlaminar differences in most parameters, whereas only some interlaminar differences were seen in NZBw/o and no differences were demonstrated in NZBw, reflecting lesser laminar differentiation in the latter. NZBw/o had a greater *bdl/btn* than NZBw in iis, greater *b1* and *b2* in iid, and tended to have greater *btn* and *tbdb*. Ectopic neurons in i differed less from ii neurons than from iii-vi neurons (but no difference was significant), and exhibited features of both iis and iid neurons. Qualitative differences in the ectopic neurons and implications of the findings will be discussed.

(Supported by NIH-NICHD 20806 and a grant from the Carl J. Herzog Foundation).

468.18

STRUCTURE & NATURE OF MEGANEURONS IN CORTICAL (MARGINAL) HETEROTOPIAS: A GOLGI STUDY. M. Marin-Padilla. Dept. Pathology, Dartmouth Med. Sch. Hanover, NH 03756.
Meganeurons have been described in cortical (marginal) heterotopias of human porencephalies (M. Marin-Padilla et al. Soc. Neurosc. Abs. 5:631, 1979; R.F. Mervis et al. Soc. Neurosc. Abs. 6:738,1980). A Golgi study of these neurons has demonstrated that most, if not all, of them represent transformed interneurons that have responded to the local alterations of the circuitry caused by the heteropia. Most pyramidal neurons within the heterotopia are not affected and their size remain within normal limits. All (45) meganeurons studied are stellate cells located within layer II or at the border between layers II and III. They are characterized by a large irregular soma from which several long dendrites originate. The dendrites extend throughout the territory of layer II and penetrate into layers I and III. All dendrites as well as the soma of these meganeurons are covered by dendritic spines. The axon arises from one of the dendrites (often at a considerable distance from the soma), is frequently ascending, and gives off many collaterals that are distributed within the territories of layers II and upper III, and some collaterals even penetrate into layer I. The nucleus of these meganeurons is quite large (as large as that of layer V pyramidal neurons) suggesting polypoidy and hence (acquired) cell hypertrophy. The presence of transformed hypertrophic interneurons in cortical heterotopias could resulted in local functional alterations. Local functional alterations could explain the brain dysfunction which is associated with dyslexias, minimal brain damage and/or epilepsy.
Supported by NIH Grant: NS22897-03

468.19

CAUDAL NERVE CONDUCTION IN THE DEVELOPING TWITCHER MOUSE AND ITS AGING HETEROZYGOUS AND NORMAL LITTERMATES.
Charles E. Olmstead. UCLA Sch. of Med., Mental Retardation Res. Center Group at Lanterman Dev. Center, Pomona, CA 91769.

The murine mutant "twitcher" is an enzymatically authentic model for the recessively transmitted human globoid cell (Krabbe) leukodystrophy. In addition to the progressive debilitation of the affected (twi/twi) animal, we have described (Behav. Brain Res. 25(1987) 143-153) subtle neurobehavioral differences between the developing heterozygous (twi/+) and normal (+/+) littermates which suggested this mutant might also be a model for heterozygote risk during life span development.

Genotype was determined by assay of galactosyloeramidase from short segments of tail. Caudal nerve sensory and motor conduction velocities were recorded with pairs of needle electrodes placed at the base, center and tip of the tail. The animals ranged in age from 10 to 590 days and were studied under Nembutal anesthesia while immobilized on a temperature-controlled platform.

As previously reported (Soc. Neurosci. Abstr. 11, 1985) the three genotypes were clearly different by 21 days of age, when at least three conduction velocity populations could be identified. As might be expected the faster NCVs were the first to go in the (twi/twi) animals, all of which were dead by 50 days of age. At 75 days of age the twi/+ mice were not significantly different than the +/+. Twi/+ animals studied at >500 days of age showed significantly more variability than their +/+ littermates.

468.20

THE RELATIONSHIP OF IMMUNE STATUS TO NEUROPATHOLOGY IN AUTOIMMUNE MICE. G. F. Sherman, P.O. Behan*, G.D. Rosen, and A.M. Galaburda. Department of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215 and Southern General Hospital, Glasgow, Scotland.

Previous studies have shown that the New Zealand Black (NZB) and BXSB mouse strains have a high prevalence of developmental cerebral anomalies (Sherman et al., *PNAS*, 82: 8072,1985, and *Acta Neuropathol.*, 74: 239,1987). These consist of ectopic collections of neurons in layer I and underlying laminar dysplasia. The anatomical characteristics of these anomalies (dated to the late stages of neuronal migration) have lead us to propose these mice as models for the similar anomalies seen in the brain of male dyslexics (Galaburda et al., *Ann. Neurol.*, 18: 222, 1985). The present study was conducted to determine whether the anatomical anomalies were seen in other immune-dysfunctional strains, to assess for immunological differences between strains possibly related to the presence of brain anomalies, and to evaluate intra-strain differences between mice with and without brain anomalies. At least 10 male and 10 female mice from strains suspected to have immune abnormalities (MRL/Mp*lpr lpr*, MRL/Mp+/+, NZB/W, DW, AKR, CBA, C58, SJL, BALB/cBy-*nu*, C57BL/6-*nu*, and additional BXSB mice) or no immune problems (BALB/c, DBA/2, and C57BL/6) were tested on a battery of immune tests (including measurements of autoantibodies, IgG, IgA, IgM, T and B lymphocyte subsets, killer cell function, and complement activation). The brains of these mice were processed in celloidin and coronal sections were cut at 35µm and stained with cresyl violet. The sections were examined under a light microscope and the incidence of anomalies was noted and the relationship to immune abnormalities analyzed. (Supported by NIH grant 20806).

NEUROENDOCRINE CONTROLS: OTHER III

469.1

COCAINE EFFECTS ON RENIN SECRETION IN CONSCIOUS RATS. J.M.Lakoski, R.E.Halpern*, K.A.Cunningham, K.Kunimoto*, and L.D.Van De Kar (SPON:G.H.Greeley). Dept. Pharmacol., Univ. Texas Med. Branch, Galveston, TX 77550.

Our present knowledge of the neuroendocrine profile of cocaine is limited. In view of the role of serotonin (5-HT) in the regulation of renin secretion and the marked effects of cocaine on spontaneous activity of 5-HT neurons recorded in the dorsal raphe nucleus, we have investigated the effects of systemic cocaine exposure on plasma renin activity (PRA) and concentration (PRC).

Male Sprague-Dawley rats received cocaine (15mg/kg, i.p.) or saline 15 min prior to decapitation. Trunk blood was collected, plasma separated, stored at -80°C, and assayed for PRA and PRC by radioimmunoassay of generated angiotensin I. Acute administration of cocaine (COC) significantly ($p<0.01$) decreased PRA and PRC as compared to saline controls (COC 5.6±0.5 vs SAL 11.0±1.0 and COC 9.5±1.2 vs SAL 16.2±0.7, respectively). Examination of the time course of this cocaine effect revealed a maximal suppression of renin secretion at 15 min in a dose-dependent manner. PRC was also evaluated in animals sacrificed 24 hr following repeated exposure to cocaine (15mg/kg, 2x daily, i.p. for 7 days) or saline. Animals treated with cocaine had elevated PRC levels as compared to saline controls (COC 9.8±1.0 vs SAL 6.1±0.6) without changes in plasma corticosterone. These observed changes in renin secretion may result from potent effects of cocaine on 5-HT neuronal systems. Supported by DA 04296.

469.2

SEROTONIN-INDUCED NATRIURESIS IS MEDIATED BY RENAL NERVES AND ANP IN THE UNANESTHETIZED RAT. R. Montes* and A.K. Johnson (SPON: I. Gormezano). Depts. of Psychology and Pharmacology and The Cardiovascular Center, University of Iowa. Iowa City, Iowa 52242.

Central serotonin (5-HT) has been proposed to be involved in the control of sodium excretion. We studied the role of renal nerves and atrial natriuretic peptide (ANP) on natriuresis induced by intraventricularly-administered (IVT) 5-HT. Animals were bilaterally renal or sham denervated and implanted with lateral ventricular cannulae. One week later, they were instrumented with femoral venous and arterial catheters and a bladder cannula. The next day, rats were infused (i.v.) continuously with a hydrating hypotonic solution, and mean arterial pressure, heart rate, diuresis, Na^+ and K^+ excretion were determined. After 150 min., 5-HT (20 µg free base) or vehicle was injected IVT. In a second experiment, plasma ANP levels were determined 15 min. after 5-HT (5, 20 µg) or vehicle. Intraventricular 5-HT produced a decrease in sodium reabsorption, consistent with a withdrawal of sympathetic tone. Renal denervation significantly attenuated the natriuretic response to 5-HT. Both doses of 5-HT caused a significant elevation of plasma ANP levels. These results suggest that the natriuretic effect produced by centrally administered 5-HT may involve neural- as well as ANP-related mechanisms.

Supported by Research Grants NIH 5PO1H1338 and Iowa Heart Association 87-G-15.

469.3

MONOAMINE RELEASE FROM THE VENTROMEDIAL HYPOTHALAMUS OF FREELY MOVING FEMALE RATS. I. Vathy and A.M. Etgen. Depts. Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Our previous study employing microdialysis in urethane-anesthetized female rats demonstrated that ovarian steroids may modulate norepinephrine (NE) release in the ventromedial hypothalamus (VMH). The present experiments measured NE release in the VMH of awake rats that were ovariectomized and steroid-treated. One day after rats were fitted with a loop-type microdialysis probe, samples were collected at 25 min intervals for 4-5 hr to measure baseline monoamine levels in a hormone-free state. Animals then received an injection of 3 ug of estradiol benzoate (EB), and two additional samples were collected to monitor possible stress effects on NE release. Twenty-four hr after EB injection, sample collection resumed for 4-6 hr; some animals received KCl stimulation in the dialysate to induce depolarization-evoked release of monoamines. During sample collection on the following day (48 hr after EB), rats were injected with 200 ug of progesterone (P) and were tested for estrous behavior with stimulus males 3.5 hr later. Preliminary results show that animals chronically implanted with dialysis probes in the VMH exhibit robust, hormone-dependent estrous behavior. We are able to detect NE in VMH dialysates of freely moving female rats, and we will present data correlating NE release with EB and P activation of estrous behavior.

469.4

ELECTRICAL COUPLING IN PRIMARY CULTURES OF ADULT RAT PINEALOCYTES. J.C. Saez*, V.M. Berthoud*, R. Dermietzel*[1], and M.V.L. Bennett (SPON: H. Buschke). Dept. Neurosci., A. Einstein Coll. Med., Bronx, NY 10461, [1]Inst. Anatomy, Univ. Essen, Essen, W. Germany

Gap junctions (GJs) mediate electrotonic and metabolic coupling. Extensive morphological studies have shown gap junctions between pinealocytes in diverse species. Here we characterize their physiological, pharmacological and biochemical properties. Female Sprague-Dawley rats (ca. 200g) were used. Anaesthetized rats were decapitated, and the pineal gland was dissected out and placed in Dulbecco's saline. After decapsulation, 2-3 glands were minced and incubated with 0.5% trypsin and 0.06% collagenase (0.17 U/mg) in nutrient medium (RPMI/F12, 1:1 Gibco) for 1 h at 37°C. The tissue was then triturated in RPMI/F12 with 10% FCS, and dissociated cells were plated on polylysine coated coverslips. After an hour, electrical coupling was evaluated in cell pairs under double current clamp. Dye coupling was evaluated by injecting Lucifer yellow into one cell of a pair or a cluster. The incidence of electrical coupling was 35%. Mean junctional conductance was 113.4 nS, range 2-400 nS, n=75. Dye coupling was seen in 30% of injected pairs (n=28) and in multiple cells of clusters. Octanol (0.4mM) rapidly and reversibly uncoupled the cells. Physiological saline also uncoupled reversibly, but more slowly consistent with removal of a nutrient factor. No immuno-labelling was seen with antibodies to the rat liver 27 kDa GJ protein, but immunoreactivity was found with an antibody specific for mouse liver 21 kDa protein. The labelling showed a spidery pattern, which is consistent with published freeze fracture micrographs of pineal GJs. Modulation of Gjs between pineal cells may be important in controlling secretion and synchronizing their circadian rhythm.

469.5

CIRCANNUAL VARIATIONS IN PINEALOCYTE SYNAPTIC RIBBONS IN THE 13-LINED GROUND SQUIRREL. J.A. McNulty*, W.A. Spurrier* and L.M Fox* (SPON: F. Lavelle). Depts. of Anatomy and Neurosurgery, Loyola Univ. Stritch School of Medicine, Maywood, IL 60153.

The synaptic ribbon (SR) is a pinealocyte organelle that is believed to play a role in the photoperiodic synthesis of melatonin. In this study, SR numbers were quantified at monthly intervals over a yearly cycle (except July) in the hibernating and seaonally reproductive ground squirrel, Citellus tridecemlineatus. Glands (4/month) of both sexes were collected during the middle of the photoperiod, processed for routine TEM and SR counted in 3 grid spaces (300 mesh) from 1 section per grid. SR numbers were high in active animals from May to Oct (35-45/10,000 um^2). During the months of Nov and Dec when squirrels were hibernating, SR frequency markedly declined (6-7 fold). There was a gradual increase in SR numbers during the period of late hibernation (Jan, Feb) and sexual maturation (Mar-May). Numerical changes in SR were due to changes in both ribbons and spherules both of which occurred either singly or in pairs. Although ribbon fields of up to 9 SR were observed, there were no consistent seasonal differences in fields comprising 3 or more SR. The winter decline in SR frequency is consistent with reports of a decrease in pineal melatonin during hibernation and supports the hypothesis that pinealocyte SR play an important role in neurotransduction of melatonin biosynthesis.

469.6

IS RHYTHMICITY IN CHICK PINEAL SYNAPTIC RIBBONS DETERMINED BY AN EXTRAPINEAL OSCILLATOR? G.N. Robertson*, D.H. Dickson*, P.C. Jackson Department of Anatomy, Dalhousie University, Halifax, N.S. B3H 4H7

In our preliminary work, 10 day old chicks yielded a 4:1 night:day difference in SR numbers, suggesting rhythmicity with a phase common to that of melatonin. To confirm rhythmicity, SRs were counted in 7-14 day old chicks (14L:10D) sampled at mid-light and mid-dark. To test for circadian rhythmicity, on day 8 of one experiment, lights were turned off for the remainder of the experiment and pineals taken on days 9 and 10. In these experiments, SR population fluctuations were found to occur only in the day 14 birds (3.4:1 night:day). We conclude that SR rhythmicity begins on, or near, day 10 post-hatch and is not directly related to the melatonin rhythm. To determine if the invasion of sympathetic fibers from the superior cervical ganglion may initiate the SR rhythm, we employed tyrosine hydroxylase immunochemistry to reveal the distribution of sympathetic fibers in the chick pineal. The percentage of innervated follicles increased markedly (day 7 - 46%, day 14 - 87%) over the critical day 10 period, suggesting that chick SR rhythmicity may be initiated and/or maintained by the sympathetic fibers (a possible reflection of an extrapineal oscillator) rather than the independent oscillators responsible for the melatonin rhythm in the chick pineal. Supported by MRC to DHD PCJ.

469.7

EPILEPTIC CONVULSIONS IN PINEALECTOMIZED MALE GERBILS. T.H. Champney. Dept. of Medical Anatomy, Texas A&M University, College Station, TX 77843.

Pinealectomy (PX) of male gerbils produces grand mal type seizures and declines in cortical norepinephrine (NE) levels within 45 minutes of the surgery. The present studies determined the role of the pineal stalk in pineal mediated convulsions, measured catecholamine levels in numerous brain regions and examined the role of PX on the sensitivity of gerbils to pentylenetetrazole (PTZ)-induced seizures. Nine week old male gerbils were PX, sham-PX or had their pineal stalk cut under metofane anesthesia. After arousal from anesthesia, stalk transection produced convulsive activity and depressions ($p<0.01$) in cortical NE levels (measured 4 hrs after surgery) to the same degree as observed in PX gerbils when both were compared to sham-PX. Depressions in NE were only observed in the parietal cortex with no alterations observed in the hippocampus, amygdala or hypothalamus. Gerbils were also PX and sham-PX under pentobarbital anesthesia (which prevents convulsive activity), allowed to recover for two weeks and injected with one of 4 doses of PTZ (15, 30, 45 or 60 mg/kg). PTZ produced increasing convulsive activity with increased dosage, and was equally effective in PX, sham-PX or control gerbils. Therefore, PX does not alter the gerbils' responsiveness to another convulsant agent, PTZ. Also, PX-induced convulsions appear to be due to an interruption of fiber tracts within the pineal stalk with a concommitant decline in cortical NE levels.

469.8

ANXIOLYTIC-LIKE ACTIONS OF MELATONIN ON A CONFLICT PROCEDURE. E.B. Naranjo-Rodríguez* and C. Reyes Vázquez. Farmacología Conductual, ENEP-Iztacala and Depto. de Farmacología, Facultad de Medicina, U.N.A.M. México 04510, D. F.

Some neuropharmacological effects exerted by melatonin, such as hypnotic, sedative and anticonvulsive actions, are very similar to those effects induced by GABAergic substances. Additionally, electrophysiological studies showed that melatonin reduces the electrical activity of several brain nuclei, also similarly to GABAergic drugs. Several of these GABAergic agents, like benzodiazepines, also induces antianxiety effects. So, if melatonin activates or produces GABAergic actions, then it also exert ansiolytic effects. Male Wistar rats were deprived of water for 48 h before testing in a licking behavior procedure. A dinkometer circuit was connected between the drinking tube of a water bottle an a grid located in the floor a plexyglass box; in such a way, that the rat completed the circuit whenever it licked the tube. Each time the subject closed the circuit, an electrical pulse of 0.5 mA and 0.1 msec, was delivered. The total number of shocks delivered in periods of 3 min, were counted electronically. Melatonin effects (1, 2 mg/kg) were compared with chlorodiazepoxide (5, 10 mg/kg), diazepam (2, 4 mg/kg) and pentobarbital (5, 10 mg/kg) effects on such procedure. Both chlorodiazepoxide and melatonin treated rats, showed the highest scores, followed by diazepam and pentobarbital, respectively. These results indicate the melatonin exert anti-anxiety effects.

469.9

EFFECTS OF MELATONIN AND IMIPRAMINE ON BRAIN SEROTONIN AND 5-HIAA OF MALE SYRIAN HAMSTERS . M. R. McCashin* and J. Vriend* (SPON: G. K. W. Cheng). Department of Anatomy, Univ. of Manitoba, Winnipeg, MB, Canada R3E 0W3.

In the present study the effects of melatonin and imipramine administration on serotonin and 5-HIAA levels were determined (by HPLC-EC) in selected brain regions of the Syrian hamster. Melatonin (25 ug sc), imipramine (0.75 mg ip), both melatonin and imipramine, or saline were administered daily for 8 weeks to male hamsters assigned to groups of 12. At the end of 8 weeks the animals were killed. Testicular weights were recorded at this time. Four animals of each group were treated with pargyline (20 mg sc) 4 hours prior to sacrifice. Serotonin levels were significantly depressed by melatonin in brain stem and in hypothalamus. Imipramine administration significantly increased 5-HIAA levels of brain stem. A small decrease in serotonin content ($p < .01$) after pargyline administration was observed only with melatonin. Melatonin administration also resulted in significant increases in hypothalamic content of the dopamine metabolites, DOPAC and HVA. Imipramine administration did not prevent the melatonin induced gonadal involution or the melatonin induced inhibition of gonadotropins. The effects of imipramine on the serotonergic system are interpreted as increased metabolism by MAO concurrent with inhibition of the reuptake mechanism.

469.10

EFFECTS OF MELATONIN AND IMIPRAMINE ON CIRCULATING LEVELS OF PROLACTIN AND THYROXIN IN PARGYLINE TREATED HAMSTERS. J. Vriend* and M. R. McCashin* (Spon J. A. Paterson). Department of Anatomy, Univ. of Manitoba, Winnipeg, MB., Canada R3E 0W3

The present study was designed to determine the effects of imipramine and pargyline on the melatonin-induced inhibition of circulating levels of prolactin (PRL) and of the melatonin-induced inhibition of circulating levels of thyroxine (T4).

Melatonin was administered daily (25 ug sc for 8 weeks) to male Syrian hamsters (N=12) alone, or in combination with imipramine (5 mg/kg ip). Additional groups of hamsters were treated with imipramine alone or with saline alone. All injections were administered 1 - 2 hrs before lights out in an animal room maintained on a 14L/10D schedule. At the end of 8 weeks the animals were killed. Four animals of each group were treated with pargyline (20 mg sc) four hours prior to sacrifice. Serum was collected for assay of T4 and PRL by RIA. As in previous studies melatonin significantly reduced circulating levels of these hormones. Four hours after pargyline a 5-fold increase in PRL was observed in imipramine treated hamsters, but not in saline or melatonin injected hamsters, nor in hamsters treated with both imipramine and melatonin. Pargyline also resulted in an increase in T4 in imipramine treated hamsters, but not in hamsters treated with imipramine and melatonin.

469.11

ZINC CONCENTRATIONS IN RHESUS BRAIN, PINEAL, AND PITUITARY. M.E. MCNEILL, J.S. MANGUM*, S.J. FIN*, S.L. LUCAS* AND J.T. BRAY*. Dept. of Anat. and Cell Biology and Shared Resources Lab. East Carolina Univ. School of Medicine, Greenville, NC 27858-4354.

Zinc (Zn) is an essential trace element in man. The requirement for Zn stems partly from its role in a variety of enzymes including those required for DNA and RNA synthesis. In the central nervous system (CNS) Zn is sequestered in specific regions, e.g., the mossy fiber pathway of the hippocampus where it appears to be associated with neurotransmission. In addition, intracranial endocrine tissue has a higher concentration of Zn than the brain as a whole, and recent studies implicate Zn as a modulator of hormone secretion. At physiological concentrations Zn suppresses the output of prolactin from dispersed pituitary cells in vitro (Judd et al., Brain Res. 294: 190, 1984). Further study of the role of Zn in endocrine-CNS interaction is warranted. Identifying regions rich in Zn is a first step to that end.

In this study brain and endocrine tissues were obtained from 7 male rhesus monkeys and freeze-dried at -38°C. Zinc concentration was determined by flame atomic absorption spectrophotometry. Concentrations in ug Zn/g dry tissue are: Temporal lobe 70 ± 4, Neurohypophysis 84 ± 30, Adenohypophysis 137 ± 59 and Pineal 162 ± 88. Endocrine tissues were found to have significantly higher concentrations than the temporal lobe, and, in addition, the adenohypophysis had a significantly higher concentration than the neurohypophysis.

469.12

SIGMA AND PHENCYCLIDINE (PCP) RECEPTORS IN RAT ENDOCRINE ORGANS. S.A. Wolfe, Jr., S.G. Culp and E.B. De Souza. Neuroscience Branch, National Institute on Drug Abuse, Addiction Research Center, Baltimore, MD 21224.

PCP and the prototypic sigma agonist N-allylnormetazocine (NANM) have been reported to alter neuroendocrine functions (stimulation of ACTH and corticosterone release and suppression of prolactin and lutenizing hormone secretion in rats). The aim of the present study was to survey endocrine organs and to identify, characterize and localize σ and PCP receptors in rat pituitary, adrenal, testis, ovary and placenta. Sigma receptors were labeled with [^{3}H]-haloperidol in the presence of 25 nM spiperone or with 1,3-di(2-[5-^{3}H]tolyl)guanidine (^{3}H-DTG), and PCP receptors were labeled using [^{3}H]-(1-[1-(2-thienylcyclohexyl)]piperidine) (^{3}H-TCP) or (+)-5-methyl-10,11-dihydro-[5-^{3}H]-dibenzo-[a,d]cyclohepten-5,10-imine (^{3}H-MK-801).

Sigma and PCP receptors were identified in endocrine tissues with kinetic and pharmacological characteristics similar to those previously reported in brain. The density of both σ and PCP receptors was highest in the testis. Pituitary, ovary and placenta had densities of σ receptors (30-70% of testis) comparable to that of cerebellum. The adrenal gland had the lowest density of σ receptors (10-20% of testis). In contrast, the density of PCP receptors, while detectable, was much lower in endocrine tissues (10-20%) than in brain. In vitro autoradiography and endocrine manipulations (e.g., hypophysectomy) are currently being used to localize and identify the cells containing PCP and σ receptors.

While the sites of action of PCP and NANM in altering hormone secretion remain to be determined, our demonstration of high densities of PCP and σ receptors in target endocrine organs provides support for peripheral sites of action of these drugs.

469.13

EVIDENCE FOR DENDRITIC AND AXONAL HORMONE RELEASE IN THE RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM. C.D. Tweedle, K.G. Smithson, & G.I Hatton. Neuroscience Program, Depts. of Anatomy & Physiology, College of Osteopathic Medicine, Michigan State University, East Lansing, MI 48824.

In order to ultrastructurally localize hormone release sites in the rat neurohypophysis the tannic acid-Ringer technique of Buma and Nieuwenhuys (*Neurosci. Lett.* 74: 151, 1987) was utilized. Three rats with normal levels of K^+ in the perfusion mixture showed only rare exocytotic figures, while 4 rats with 50 mM K^+ (substituted for Na^+) in the perfusion mixture showed frequent exocytotic figures. Of 20 such figures per rat, 86.3 ± 2.4 % were at morphologically identified neurosecretory endings containing microvesicles and contacting the basal lamina. This supports our contention that the observed changes in the size and number of such endings are relevant to hormone release.

Upon examination of the supraoptic nuclei from the same animals it was found that in the rats with high K^+ occasional exocytotic figures of dense core granules occurred in dendrites, but not in somata. This apparent dendritic release could accomplish local information transfer and/or be a source of the oxytocin and vasopressin found in the CSF. Current experiments are underway to see if similar findings are obtained with more physiological stimulation of hormone release. Supported by NIH grant NS09140 and by a MSTP fellowship to KGS.

469.14

ß-ADRENERGIC STIMULATION DECREASES GLIAL, AND CONCOMITANTLY INCREASES NEURAL, CONTACT WITH THE BASAL LAMINA IN RAT NEUROINTERMEDIATE LOBES INCUBATED *IN VITRO*. K.G. Smithson, I. Suarez* & G.I. Hatton. Neuroscience Program & Physiology Dept., College of Osteopathic Medicine, Michigan State University, East Lansing, MI 48824

Isoproterenol induces a change from flattened to stellate morphology in cultured adult pituicytes. We sought to determine if similar changes could also be observed in the isolated neural lobe. Neurointermediate lobes from adult male rats were isolated and placed in chambers containing artificial CSF at 37°C. Treatment consisted of a 15 min. incubation with CSF containing either 0.2% ascorbate or isoproterenol (10^{-7} or 10^{-8} M) with 0.2% ascorbate. Tissue was then prepared for conventional electron microscopy after which morphometric evaluation of the percent of, pituicyte and neurosecretory, membrane contact with the basal lamina was performed. Isoproterenol stimulation resulted in a significant decrease in pituicyte and increase in neural contact with the basal lamina, as compared to control. These changes are similar to that observed during conditions which require increased hormone release (e.g. dehydration or lactating). These results suggest that ß-agonist mediated changes in pituicyte morphology may represent a mechanism underlying these phenomena. Supported by NIH grant NS09140 and by a MSTP fellowship to KGS.

469.15

INHIBITORY EFFECT OF NOREPINEPHRINE (NE) ON THE ELECTRICAL ACTIVITY OF CAUDALLY-PROJECTING PARAVENTRICULAR (PVN) NEURONS IN RAT. Y.I.Kim, C.A. Dudley and R.L. Moss. Dept. of Physiology, Univ. of Texas Southwestern Medical Center, Dallas, Texas 75235.

Utilizing conventional extracellular single unit recording techniques, the effect of iontophoretically applied NE on the activity of caudally-projecting PVN neurons was assessed in male Sprague-Dawley rats anesthetized with urethane. These PVN neurons were identified antidromically by electrical stimulation (50 uA - 1.5 mA) of the caudal ventrolateral medulla (mean ± SEM antidromic latency: 34.7 ± 2.4 ms). In 15 of 19 identified PVN neurons (mean ± SEM baseline firing rate: 2.7 ± 0.6 impulses/s), NE was demonstrated to be inhibitory. No excitatory NE effect was observed. Five of those 15 neurons were also antidromically identified as projecting to the posterior pituitary (mean ± SEM antidromic latency: 15.0 ± 2.5 ms). The inhibitory action of NE was selectively blocked by the alpha antagonist, phentolamine (n=7) that was co-iontophoresed with NE, but not by the beta antagonist, timolol (n=9). In contrast to the inhibitory action of NE on the caudally-projecting PVN neurons, an excitatory action of NE was frequently observed in the PVN neurons projecting only to the posterior pituitary: out of 20 such neurons tested, 15 were excited and 3 were inhibited by NE.

The present results strongly suggest that NE exerts an alpha-receptor-mediated, specific inhibitory effect on the PVN neurons projecting to or passing through the caudal ventrolateral medula. These PVN neurons include a subpopulation of neurons which also project to the posterior pituitary. Supported by HD09988-V.

469.16

PARABRACHIAL NUCLEUS INVOLVEMENT IN THE INHIBITION OF DEHYDRATION-INDUCED VASOPRESSIN RELEASE BY WATER INTAKE. L.E. Ohman, R.E. Shade and J.R. Haywood. Dept. of Pharm., The Univ. of TX Hlth. Sci. Ctr., San Antonio, TX.

High circulating levels of vasopressin (pAVP) induced by water deprivation (WD) decrease within minutes of water ingestion. The lateral parabrachial nucleus (LPBN), known to be involved in the regulation of water intake and AVP release, may contribute to this inhibition of WD-induced pAVP. Rats were given either bilateral electrolytic or sham tract lesions of the LPBN. Water was removed for 48 hr, after which the rats were allowed to drink for 1 hr. Plasma osmolality (Osm) and AVP were determined prior to WD, before access to water, and 10 min after the onset of drinking. Plasma Osm increased from 289±3 to 298±2. mOsm in lesioned rats and from 288±3 to 296±2 mOsm in sham lesioned rats in response to 48 hr WD. Plasma AVP levels also increased in both groups (Lesion: 1.0±.3 to 13.5±2.4 μU/ml; Sham: 0.7±0.2 to 15.4±3.4 μU/ml). Lesioned rats drank significantly more water (18.6±1.5 ml) than sham rats (11.5±0.6 ml), but pOsm decreased to a similar value in both groups (Lesion: 284±5 mOsm; Sham: 291±2 mOsm). Plasma AVP decreased 10.6±1.4 μU/ml (75%) in sham lesioned rats after water intake, while the pAVP decrease in lesioned rats, 5.7±1.5 μU/ml (45%), was significantly less. These data suggest that the LPBN partially mediates the inhibition of dehydration-induced pAVP release by water intake. (Supported by HL32977).

469.17

IMPAIRED GLUCAGON SECRETION IN DIABETIC AND GALACTOSEMIC RATS MAY INVOLVE VAGAL-CHOLINERGIC NEUROPATHY. K.A. Skau & D.G. Patel*. Div. Pharmacol. & Med.Chem., U. Cincinnati, Cincinnati, OH 45267.

Glucagon secretion in response to insulin hypoglycemia is impaired in diabetic patients and rats and this impairment correlates with diabetic neuropathy. We have recently shown that glucagon is secreted in response to direct vagal nerve stimulation and that this response is also impaired in diabetic rats (FASEB J. 2: A1594, 1988). The present study expands on the characteristics of vagal-stimulated glucagon secretion in galactosemia and diabetes. Rats were anesthetized with chloral hydrate (350 mg/kg i.p.) and the carotid artery was cannulated for blood sampling. Vagus nerves were stimulated via silver electrodes for varying times at fixed frequency (20 Hz). Hypoglycemia-induced glucagon secretion was produced by constant infusion of insulin, via the jugular vein, until plasma glucose levels dropped to 40 mg/dl. Plasma glucose and glucagon levels were determined by glucose oxidase and RIA respectively. Plasma glucagon levels increased with increasing durations of vagal stimulation. This vagal-stimulated, as well as hypoglycemia-induced, secretion was totally prevented by atropine pretreatment (2 mg/kg i.v.). Furthermore, carbachol infusion resulted in elevated plasma glucagon. Glucagon secretion was increased by either right or left vagus stimulation. Although insulin-induced glucagon secretion was prevented by atropine it was not eliminated by acute bilateral vagotomy. In addition to diabetes, galactose-fed rats exhibited a similar impairment of glucagon secretion. It is concluded that glucagon secretion is regulated, in part, by cholinergic influences that may involve the vagus nerve, but may also involve other systems. (Supported by U. Cincinnati Research Council.)

469.18

METABOLIC MAPPING OF THE DORSAL VAGAL COMPLEX IN DEHYDRATED RATS. S. Shaver, M. Kadekaro[1] and P.M. Gross. Neurosurg. Res. Unit, Queen's Univ., Kingston, Ont. and [1]Div. Neurosurg., Univ. Texas Med. Branch, Galveston, TX.

Receptors and immunoreactive perikarya for angiotensin II (AII) are populated densely in neurally-linked subunits of the medullary dorsal vagal complex (DVC) - the area postrema (AP), nucleus of the solitary tract (NTS), and dorsal motor nucleus of the vagus nerve (DMN). We hypothesized that metabolic activity in DVC would be stimulated by dehydration associated with high systemic AII. We applied the [^{14}C]deoxyglucose method in conscious Sprague-Dawley (SD) rats, SD rats deprived of water (WD) for 120 h, Long-Evans (LE) rats, Brattleboro (DI) rats, and DI rats WD for 18 h. Values for glucose metabolism (GM) in control SD rats were AP (.69±.03 umol/g/min), NTS (.60±.04), and DMN (.56±.04); similar values were found in LE rats. WD in SD rats increased GM of AP, NTS, and DMN by 38%, 35% and 21%, respectively. In DI and WD-DI rats, GM increased by 12 to 39% in AP and NTS; no change occurred in DMN. WD-DI rats had higher GM in AP and NTS than water-sated DI rats. We speculate that AII-enriched regions within DVC are activated by circulating AII via the AP in these rat models of dehydration.

469.19

TOPOGRAPHY OF CAPILLARIES AND GLUCOSE METABOLISM WITHIN SUBREGIONS OF RAT AREA POSTREMA AND SUBFORNICAL ORGAN. J.J. Pang*, N.M. Sposito*[1], S.W. Shaver, M. Kadekaro[2] and P.M. Gross (SPON: F.A. Kutyna) Neurosurg. Res. Unit, Queen's Univ., Kingston, Ont., [1]Dept. Neurosurg, SUNY-Stony Brook, NY, and [2]Div. Neurosurg., Univ. Texas Med. Branch, Galveston, TX.

Neuronal circumventricular organs (CVOs; eg, area postrema, AP, subfornical organ, SFO) are organized in subregions defined by cytoarchitecture, afferent terminals, and distribution of neuroactive substances. In adult albino rats, we examined whether capillary density (CD) and parenchymal glucose metabolism (GM), which are closely coupled in nervous tissue, conformed topographically in these CVOs. CD was assessed by computer processing of micrographic sections (x240) from aldehyde-fixed brains. [^{14}C]deoxyglucose and image analysis were used to measure GM. In coronal sections (obex - 0.25 mm), AP subdivided into 3 zones having CDs of 200/mm^2 (dorsal) to 407/mm^2 (medial) for vessels ≤ 7.5 um; corresponding GMs were 0.52 to 0.69 umol/g /min, respectively. In SFO dorsal and ventromedial zones, CD and GM correlated directly throughout the rostrocaudal axis. Differential CD and GM within neuronal CVOs further identify subregions that may be distinct functionally.

470.1

HYPOTHALAMIC MODULATION OF PERIPHERALLY-INDUCED JAW CLOSURE IN THE CAT. S. Weiner*, A.B. Shaikh* and A. Siegel. Depts. of Restorative Dentistry and Neurosciences, UMDNJ, Newark, New Jersey 07103.

The lateral hypothalamus can modulate jaw closure, but little is known of the interaction between peripheral receptors and hypothalamus in regulating this response. We observed the effects of peripheral stimulation of the periodontal ligament upon the magnitude of masseteric EMG activity in the presence of hypothalamic stimulation.

Hypothalamically elicited quiet biting attack (QBA) and affective defense (AD) sites were identified and bipolar surface electrodes were attached bilaterally over the masseter muscles. EMG recordings were compared under conditions involving paired trials of tooth probing alone and in combination with hypothalamic stimulation. Tooth probing alone predominantly increased ipsilateral EMG activity. In contrast, dual stimulation involving tooth probing coupled with activation of contralateral QBA sites predominantly increased EMG activity on the side contralateral to tooth probing. Stimulation of AD sites did not show this effect. Moreover, stimulation of QBA and AD sites elevated overall EMG activity in comparison with non-behavioral (control) sites in the hypothalamus.

We suggest that this experimental paradigm can serve as a model for further study of the mechanisms by which the hypothalamus regulates jaw muscle activity. **[Supported by the Foundation of UMDNJ and NIH Grant NS07941-19].**

470.2

IMMUNOHISTOCHEMICAL DIFFERENTIATION OF ELECTROPHYSIOLOGICALLY DISTINCT NEURONS IN THE REGION OF THE RAT PARAVENTRICULAR NUCLEUS. N.W. Hoffman*, J.G. Tasker and F.E. Dudek. Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024

Neurons with low-threshold calcium spikes (LTS) have been found immediately lateral to the hypothalamic paraventricular nucleus (Poulain, P. and Carette, B., Brain Res. Bull. 19:453, 1987). These neurons are distinguishable from non-LTS neurons in the nucleus, not only by the presence of LTS potentials, but also by their afterhyperpolarizations, current-voltage relations and baseline synaptic activity (Tasker, J.G. and Dudek, F.E. Soc. Neurosci. Abstr. 13:1370, 1987). After electrophysiological characterization, LTS and non-LTS neurons were injected with Lucifer yellow and neurophysin immunohistochemistry was performed. Preliminary results showed that 7 of 7 Lucifer-labeled LTS neurons were neurophysin-negative and that 1 of 1 non-LTS neuron was neurophysin-positive. LTS neurons were found to surround the nucleus. From electrical and immunohistochemical data, non-LTS neurons appear to be magnocellular neuropeptidergic cells, whereas LTS neurons probably are not. These preliminary findings support the hypothesis that the two cell types are both physiologically and anatomically distinguishable. Supported by AFOSR-87-0361, BNS 87-96288 and NS 8049.

470.3

LOCAL CIRCUIT INTERACTIONS BETWEEN NEURONS IN THE REGION OF THE RAT PARAVENTRICULAR NUCLEUS. J.G. Tasker and F.E. Dudek, Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024

Local synaptic interactions in the supraoptic and paraventricular nuclei (PVN) have been postulated on the basis of electrophysiological studies using electrical stimulation to activate presynaptic neurons. This technique stimulates not only local neurons, but also axons projecting from other parts of the brain. We have used glutamate microapplication to stimulate presynaptic neuronal somata and dendrites without activating axons of passage. Neurons in the PVN were recorded intracellularly while glutamate drops (10-100 mM, 50-250 μm in diameter) were pressure-applied to several locations around the PVN. Saline drops were applied through a second barrel of the stimulating pipette to control for mechanical effects of drug application. Preliminary results showed that glutamate application, when effective, elicited an increase of EPSP's and/or IPSP's in the recorded neuron. Higher concentrations of glutamate caused trains of PSP's lasting up to several seconds. Picrotoxin (50 μM) blocked the IPSPs, suggesting they were mediated by $GABA_A$-receptors. These findings provide electrophysiological evidence for local synaptic interactions in the region of the PVN with a technique that is unlikely to involve activation of axons of passage. Supported by AFOSR-87-0361, BNS 87-96288 and NS 8049.

470.4

KYNURENATE ANTAGONISM OF FAST EPSPs IN PARAVENTRICULAR NEURONS. J.-P. Wuarin* and F.E. Dudek (SPON: W.B. Macklin). Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024

Excitatory amino acids (EAAs) may play a major role in fast synaptic transmission within the hypothalamus. Electrophysiological evidence for their involvement in the rat supraoptic nucleus has been provided (Gribkoff, V.K. and Dudek, F.E., Brain Res. 442:152, 1988). We have undertaken preliminary studies in the paraventricular nucleus (PVN) to test this hypothesis. Intracellular recordings (n=4) were obtained from guinea-pig hypothalamic slices in 50 μM picrotoxin. Electrical stimuli dorsal to the fornix evoked excitatory postsynaptic potentials (EPSPs). The EAA antagonist, kynurenate, was bath applied; EPSPs were averaged every 5 min for 20-30 min in steady state. The mean decrease was 5% for 100 μM, 44% for 300 μM and 68% for 1 mM (n=2 each). The effects were reversible (recovery within 45 min after 300 μM). From the electrophysiological properties, one neuron appeared to be a magnocellular neuropeptidergic cell, but three others had low-threshold calcium spikes (Poulain, P. and Carette, B., Brain Res. Bull. 19:453, 1987). These data support the hypothesis that EAAs are an important class of transmitter for fast synaptic actions throughout the hypothalamus. Supported by AFOSR-87-0361 and BNS 87-96288.

470.5

IN VIVO MICRODIALYSIS STUDIES OF NOREPINEPHRINE (NE), 3,4-DIHYDROXYPHENYLETHYLENEGLYCOL (DOPEG), VASOPRESSIN (VP) AND OXYTOCIN (OT) IN THE ANTERIOR HYPOTHALAMUS. D. Nakahara*†, E. Kogosov*, F. Ross-Cisneros*, M. Morris*#, T. Nagatsu*† and N. Alexander. †Nagoya Sch. of Med., Nagoya, Japan, #Bowman Grey Sch. of Med., Winston-Salem, NC 27103 and Univ. of So. Calif. Sch. of Med., Los Angeles, CA 90033.

Brain microdialysis is a technique for sampling extracellular fluid components from freely moving rats (Amer. J. Physiol. 254:R396, 1988). For the present study, a dialysis probe was inserted into the anterior hypothalamus through a guide tube implanted stereotaxically 1 week earlier (n=14). A small dialysis bag (2 mm long, 0.2 mm diam., 5000 mw cutoff) at the tip of the probe, was perfused with Ringer's solution (2 ul/min) and molecules from surrounding tissue diffused into the bag. Dialysate samples were collected at 30 minute intervals for 5 hours as the rat moved or slept. Recovery, measured in vitro, is about 20% and is independent of concentrations outside the bag. Basal dialysate concentrations were (pg/ml): NE, 80.6±15 and DOPEG, 425±57. Pargyline, added to perfusate (0.1 mM and 1.0 mM) produced a dose-related effect on DOPEG and NE. The maximum effects equaled those of I.P. pargyline (100 mg/kg), namely, a reduction of 50% in DOPEG and a 4-fold increase in NE in the dialysate. VP and OT, in pooled samples extracted on Sep Pak, showed a dose-related immuno-reactivity; their average levels were 2.5 and 4.7 pg/ml, respectively. Basal levels of VP, measured directly in 100 ul dialysate samples was 2.0±0.38 pg/ml (n=9). These findings show that brain microdialysis can provide new information on mechanisms regulating neurotransmitter and neuropeptide processes in the anterior hypothalamus.

470.6

ASYMMETRIC HYPOTHALAMIC MONOAMINE METABOLISM IS RELATED TO SIDE OF OVULATION IN LIZARD. P.H.Desan, R.E.Jones*, K.H.Lopez* and H.B.Austin*. Dept. of Neurology, Stanford Med. Ctr., Stanford, CA 94305, and Dept. of EPO Biology, Univ. of Colorado, Boulder, CO 80309.

The American chameleon (Anolis carolinensis), like certain other lizards, apes and humans, alternates ovulation between the left and right ovaries. We measured NE, MHPG, DHPG, DA, DOPAC, 5HT and 5HIAA using HPLC with serial oxidative-reductive electrochemical detection in microdissected left and right diencephalon from 15 lizards collected during their first spring ovulation cycle (8 had a maturing follicle on the right side and 7 on the left). Of the calculated neurotransmitter metabolite/parent neurotransmitter ratios, only DOPAC/DA and 5HIAA/5HT were significantly different between the two sides, being lower on the maturing follicle side than on the quiescent side: DOPAC/DA = 0.49 ± 0.06 vs. 0.61 ± 0.05, and 5HIAA/5HT = 1.14 ± 0.10 vs. 1.32 ± 0.10; paired t-test significance for both comparisons $p < 0.05$). On an individual basis 13 of 15 animals showed a lower DOPAC/DA ratio on the maturing follicle side than on the quiescent side, and 11 of 15 showed a lower 5HIAA/5HT ratio. These observations indicate that an asymmetry of hypothalamic monoamine metabolism controls or is controlled by the side of the follicle growth, perhaps via hypothalamo-medullary interconnections and the vagal innervation of the ovaries.

470.7

PROPERTIES OF ORGANUM VASCULOSUM LAMINA TERMINALIS (OVLT) NEURONS IN THE RAT. L.P. Renaud, R. Nissen* and C.W. Bourque, McGill Univ. Centre for Research in Neuroscience, Montreal, Canada H3G 1A4.

The OVLT, a circumventricular structure involved in hydromineral homeostasis, contains cells anatomically linked with the nucleus medianus (NM), supraoptic (SON) and paraventricular (PVN) nuclei. However, there is scant information as to their electrical properties. Intracellular recordings obtained from 42 OVLT neurons in slices and superfused explants of rat basal forebrain revealed average resting membrane potentials near -65mV, input impedances of 80-280 MΩ, linear V-I plots below resting membrane potentials and low threshold calcium spikes. Antidromic activation from the region of NM, PVN and/or SON agreed with axon branching and their trajectories as visualized in Lucifer-yellow filled cells. The latter also revealed a simple cellular morphology (soma 10-15 µm, 0-1 dendrites). Each of 17 tested cells depolarized in hyperosmotic (+15 mosmol) media. These data suggest an intimate relationship of OVLT neurons with forebrain areas regulating body fluid balance. Supported by FCAR, QHA and MRC.

470.8

GASTROINTESTINAL PATHOLOGIES INDUCED IN RATS BY HYPOTHALAMIC LESIONS. **N.I. Wiener, P. Wright*, J. Andersen*. Department of Psychology, York University, Toronto, Canada, M3J 1P3.**

Gastrointestinal pathologies were measured in rats as a consequence of bilateral anodal electrolytic and bilateral neurotoxic kainic acid lesions of the paraventricular nucleus of the hypothalamus. Both manipulations induced a high level of gastric ulceration within 24 hours. However, kainic acid also produced severe duodenal ulceration. The kainic acid induced duodenal ulcers appeared similar to those produced by cysteamine and other duodenal ulcerogens. The duodenal ulcers were located on the anterior and/or posterior wall of the proximal duodenum near the pylorus.

Subcutaneous injection of phentolamine and propranolol facilitated the development of gastric ulcers. Subcutaneous injection of haloperidol facilitated duodenal ulceration.

These data support the hypothesis that gastric ulcers result from depletion of central noradrenergic stores and duodenal ulcers from a reduction in dopaminergic activity in or near the paraventricular nucleus of the hypothalamus.

470.9

CHARACTERISTICS OF THERMOSENSITIVE AND OSMOSENSITIVE NEURONS IN THE RAT DIENCEPHALON. K.A. Travis* and J.A. Boulant, Department of Physiology, College of Medicine, The Ohio State University, Columbus, Ohio 43210.

Hypothalamic neurons respond to various homeostatic challenges, including changes in temperature and osmolarity. This study evaluated diencephalic neuronal thermosensitivity and osmosensitivity in Sprague Dawley, Wistar-Kyoto, and spontaneously hypertensive rats. In vitro neuronal activity was recorded from several nuclei in horizontal tissue slices perfused with control (300 mOsm/kg), hyposmotic (280 mOsm/kg) and hyperosmotic (320 mOsm/kg) media. The majority of neurons responded to at least one test medium. Temperature sensitive neurons showed no specific pattern of response to the two test media. Temperature insensitive neurons tended to be unresponsive in the hyperosmotic medium, and either increased or showed no change in the hyposmotic medium. While there were no differences in the responses of hypertensive and normotensive rats, there were differences in the responses of temperature sensitive and insensitive neurons to changes in osmolarity. (Supported in part by NSF, AHA and NIH grants.)

470.10

POSTNATAL MSG TREATMENT ALTERS PROSTAGLANDIN INDUCED FEVER IN RATS. S.M. Martin, T.J. Malkinson, L. Bauce, W.L. Veale and Q.J. Pittman. Biology Dept, Mt.St.Vincent Univ, Halifax, NS B3M 2J6 and Neurosci. Res. Group, Univ of Calgary, Calgary, AB, T2N 4N1 Canada.

Alpha-melanocyte stimulating hormone (α-MSH) has been implicated as an endogenous antipyretic active during the rising phase of fever. Since brain α-MSH is synthesized in the arcuate region, we hypothesized that lesioning of this area, with monosodium glutamate (MSG), would reduce the levels of α-MSH and alter the fever response induced by central injections of prostaglandin (PGE_1). Wister pups were given randomly either ip injections of MSG (4 mg/kg body wt) or equal volumes of saline on days 2,4,6,8,10 after birth. At maturity, male rats were implanted with intraventricular (ICV) cannulae and abdominal temperature transmitters. After recovery from surgery, the animals were given ICV injections (PGE_1, (20 ng) and body temperature data were computer collected every 5 min for 2 h before and 4 h following injections. After completion of the experiments brain tissue was assayed for α-MSH. The results show that the fever in the MSG treated animals rose faster and was higher ($p<0.05$) than fever in the saline group during the first 20 mins. As MSG pretreatment decreases α-MSH levels in brain, the enhanced fever in the MSG treated animals supports the hypothesis that α-MSH is an endogneous antipyretic. (supported by Mt.St.Vincent and the MRC).

470.11

PERFUSATE PO_2 AND NEURONAL THERMOSENSITIVITY IN PREOPTIC AREA (POA) SLICES. M. Shibata and C.M. Blatteis. University of Tennessee, Memphis, TN 38163.

In electrophysiological studies, it is usual to gas the perfusates of brain slices with $95\%O_2/5\%CO_2$, whereas $21\%O_2/5\%CO_2$ is normally used to maintain brain tissue cultures. To determine whether $95\%O_2$ might be needlessly high, possibly affecting neuronal excitability, we compared the effects of the 2 gases on the firing rates (FR) of thermosensitive neurons in 350-µm thick slices from guinea pigs POA perfused at 1 ml/min with artificial cerebrospinal fluid (ACSF) gassed with $95\%O_2$/ or $21\%O_2$/ $5\%CO_2$. Thermosensitivity (Q_{10}) was assessed by FR changes with perfusate temperatures (T_p=32-42°C), according to established criteria. Results: 1) Within 1.5 h, incubation chamber perfusate P_{O_2} at T_p 37°C reached 300-350 mmHg when gassed with $95\%O_2$, and 130-160 mmHg with $21\%O_2$. 2) Of 7 thermosensitive units initially characterized in $21\%O_2$-ACSF, 4 units lost their thermosensitivity completely, 2 decreased their Q_{10} greatly, and 1 ceased spontaneous firing when $95\%O_2$-ACSF was substituted. 3) By contrast, the Q_{10} of 6 thermosensitive units originally identified in $95\%O_2$-ACSF was not affected when the perfusate was switched to $21\%O_2$-ACSF. 4) Generally, initial Q_{10} values measured in $21\%O_2$- were higher than in $95\%O_2$-ACSF. These findings suggest that oxygenating with $95\%O_2/5\%CO_2$ increases the perfusate P_{O_2} to an unphysiological range and may cause impairment of POA neuronal responses to thermal stimuli.

470.12

HYPOTHALAMIC REGULATION OF MALE REPRODUCTIVE BEHAVIOR AND ANALGESIA IN THE RAT. M.R. Sharpe*, R.A. Leslie and D.M. Nance. Anatomy Dept., Dalhousie Univ., Halifax, N.S., B3H4H7, Canada.

Responses to noxious and sexual stimuli have visceral components which are modulated by opiates. Although the VMH is known to be involved in the control of both pain and male sexual behaviour, the role of the PVN has not been tested despite its role in autonomic function. Therefore we examined the role of the ventromedial hypothalamus (VMH) and the paraventricular nucleus (PVN) in the dual control of analgesia and sexual behavior. Bilateral lesions were made in the VMH or PVN of male rats with ibotenic acid and the effects of saline, naloxone (4 mg/kg) and morphine (10 mg/kg) on the analgesic threshold to heat (tail-flick test) and pressure (pressure algometer) were tested. Drug treatments were repeated and the effects on male sexual behavior tested. There was a significant and modality-specific effect of the lesions on analgesia. The PVN group was hyperalgesic to heat whereas the VMH group was hypoalgesic to heat, relative to controls. The PVN group was hyper-sensitive to morphine and naloxone on the tail-flick test and the VMH group was hypo-sensitive to these drugs. There was a modest reduction in mounting behavior in the PVN group and a small increase in the VMH group, relative to controls. Morphine reduced male sexual behavior in all groups, but the effect was smaller in the PVN rats. The PVN group showed a dramatic increase in mounting behavior following naloxone treatment, whereas naloxone had no effect on the VMH and control rats. Thus lesions in the VMH and PVN have opposite effects on both male sexual behavior and pain and the effects on analgesia are modality-specific. The drug effects indicate that the lesions produce an alteration in opioid systems, and these alterations in turn exert opposite effects on both sexual behavior and pain. The results after PVN lesions further suggest that the ANS may play a role in the regulation of male sexual behavior and the response to pain. Supported by M.R.C.

470.13

Developmental Regulation of Hypothalamic and Pituitary Aromatase Activity in the Male Rat. E.D. Lephart*, and S.R. Ojeda (Spon: S. Kiser). Dept Physiol, Univ TX SW Med Ctr, Dallas, TX 75235, and Div Neurosci, OR Reg Pri Res Ctr (SRO), Beaverton, OR 97006.

In the adult male rat brain aromatase activity is regulated by androgens. We have investigated whether dihydrotestosterone (DHT) regulates hypothalamic and/or pituitary aromatase activity prior to puberty. Two-, 5 and 8-wk- old male rats were divided into three groups: intact controls, castrated for 2 weeks, and castrated-treated with 20 mg DHT/kg body wt. At 28-(juvenile), 48- (peripubertal) and 68- (post-pubertal) days of age the hypothalamus and anterior pituitary were incubated with [1α, 1β-^{3}H] testosterone (T). Aromatase activity was estimated by measuring the release of [1β-^{3}H] from T into water. The enzyme activity decreased markedly in the pituitary and hypothalamus of postpubertal rats. Castration increased the activity in the pituitary at all ages tested and DHT treatment suppressed the increase. The response of hypothalamic aromatase to castration and castration plus DHT replacement was opposite to that of the pituitary. The results indicate that aromatase activity is regulated differently by androgens in the hypothalamus and pituitary during sexual development and suggest that the post-pubertal decrease in pituitary enzyme activity can be attributed to androgens while the decline in hypothalamic enzyme activity is androgen-independent.

470.14

SUPRACHIASMATIC NUCLEUS EXPRESSION OF GENE PRODUCT INDUCED BY NGF IN PC12 CELLS. A.N.van den Pol, C.Decavel*, A. Levi*, B.Paterson*. Sect.Neurosurgery, Yale Med. Sch.,New Haven,Ct.06510; Lab Biochem. NCI,NIH, Bethesda, 20892.

NGF induces in PC12 cells a 50 fold enhancement of mRNA coded for by the VGF gene (Science,229:393). Two different restriction fragments from a VGF cDNA clone were fused into the beta-galactosidase (B-gal) bacterial gene. Antisera made in rabbits against each of the two fusion products stained neurons in the rat suprachiasmatic nucleus (SCN) intensely. In addition to diffuse cytoplasmic staining, intrinsic and projection axons of the SCN were labeled. The expression of the VGF related protein was found in the dorsomedial area of the SCN where cells also contain vasopressin, and in SCN of Brattleboro rats lacking the vasopressin gene. Perikarya, but not axons, of the magnocellular paraventricular and supraoptic nucleus showed some weaker positive immunostaining. A low level of immunoreactivity was also seen in some other non-hypothalamic brain areas. SCN immunostaining was not blocked by bacterial B-gal lysate absorption; different antisera made against the product of an unrelated cDNA fusion to B-gal showed no SCN immunostaining. Adult SCN expressed no immunoreactivity for NGF-receptor, while control cells of the medial septum did. These results indicate that a previously unidentified protein coded by the VGF gene is strongly expressed in a subpopulation of SCN cells and axons, and that this protein may not require NGF for a strong expression in the CNS.

470.15

INTRACELLULAR LABELING OF ELECTROPHYSIOLOGICALLY IDENTIFIED NEURONS IN THE TUBERAL PORTION OF THE SUPRAOPTIC NUCLEUS. B. Smith* and W.E. Armstrong. Dept of Anat. Neurobiol., Univ. Tenn., Memphis, The Health Science Center, Memphis, TN, 38163.

The tuberal, or retrochiasmatic, portion of the supraoptic nucleus (SONt) consists largely of vasopressin neurons whose dendritic and axonal morphologies have not been described. SONt neurons impaled in hypothalamo-neurohypophysial explants in vitro were filled with biocytin (Horikawa and Armstrong, J. Neurosci. Meth, in press) and reconstructed in serial sections. All injections were made into neurons from which stable recordings were obtained and whose input resistance (mean = 124 MΩ) and response to neural stalk stimulation (mean constant latency = 6.75 msec) had been recorded.

SONt neurons possessed 2-4 sparsely branching, varicose dendrites which were largely oriented in the horizontal plane. Spines were occasionally observed, emitting from both somata and dendrites. The beaded axon arose from either the soma or a primary dendrite, and coursed in a wide dorsomedial arc before turning caudally into the neurohypophysial tract. In some cases axons were traced as far as 2 mm from the soma, almost reaching the neural stalk. Short, hair-like appendages arising from axonal dilatations were fairly common, but long collaterals were not observed. SONt neurons displayed a prominent afterhyperpolarization and firing frequency adaptation which distinguished them from a more dorsal group of neurons which fired rapidly and displayed low-threshold, slow action potentials, but which could not be stimulated antidromically. Supported by NIH grant NS23941 (WEA).

470.16

FLOW CYTOMETRIC ANALYSIS OF STIMULUS-INDUCED DEPOLARIZATION IN DISSOCIATED EMBRYONIC RAT HYPOTHALAMIC NEURONS S. M. O'Connell*, J. P. Grierson, R. E. Petroski and H. M. Geller. (SPON: G. S. F. Ling) Department of Pharmacology, U.M.D.N.J. - Robert Wood Johnson Medical School and The Graduate School, Rutgers University, 675 Hoes Lane, Piscataway, NJ 08854.

Two classes of voltage-sensitive fluorescent dyes are known which respond to changes in the cell membrane potential by varying their intensity of emission. In the case of the cyanine dye $DiOC_5(3)$, cell depolarization produce a drop in fluorescent output. We have used this dye to examine stimulus-induced depolarization in fetal hypothalamic neurons, obtained from embryonic day 17 rats. Neurons were dissociated by enzymatic treatment followed by mechanical dispersion and the cells loaded with 20 nM $DiOC_5(3)$ at a concentration of 10^6 cells/ml. The addition of KCl (25 - 100 mM) and veratridine (50-100 uM) depolarized the majority of these cells. Analysis of fluorescence with time revealed that a large proportion of cells begin to repolarize after 5-6 minutes, bringing their mean resting membrane potential close to pre-stimulation levels. The glutamate analog, NMDA (10 uM), also depolarized 40-50% of cells. An interesting corollary to these experiments is a K^+-induced cell swelling, which does not appear to be attributable to chloride concentration nor changes in osmolality. Parallel studies of stimulus-induced calcium mobilization with these cells will be presented in another presentation (Grierson et al). Supported by NIH NS25168 and a grant from the UMDNJ Foundation.

HYPOTHALAMUS II

471.1

LIGHT- AND ELECTRON MICROSCOPIC STUDY OF BASAL FOREBRAIN NEURONS PROJECTING TO NUCLEI GEMINI AND THEIR RELATION TO VENTRAL PALLIDUM AS DEMONSTRATED BY SP-IMMUNOHISTOCHEMISTRY. L. Heimer and D. S. Zahm. Departments of Otolaryngology and Neurosurgery, University of Virginia, VA 22908 and Dept. of Anatomy, St. Louis University, MO 63104.

Basal forebrain neurons were retrogradely labeled with WGA-HRP or Fluoro-Gold following injection of the tracer in the nuclei gemini of the posterolateral hypothalamus. The labeled cells are located among the heavily myelinated medial forebrain bundles deep to the multiform layer of the olfactory tubercle. The distribution of labeled cells extends from the rostral pole of the olfactory tubercle into the nucleus of the horizontal limb of the diagonal band at the caudal pole of the tubercle, and in a plane approximately parallel to the ventral surface of the brain. Where this plane intersects the ventral pallidum, the labeled neurons freely mingle with ventral pallidal elements. The labeled cells are large and polygonal with large radiating dendrites which are related, at least along their proximal segments, to a moderately large and heterogenous population of boutons making symmetrical or asymmetrical synaptic contacts.

471.2

CYTOARCHITECTURE AND EFFERENT CONNECTIONS OF THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS OF SYRIAN HAMSTERS. Robert L. Meisel, Mark R. Sterner, and Vickie R. Luttrell. Dept. of Psychological Sciences, Purdue Univ., West Lafayette, IN 47907.

The ventromedial nucleus of the hypothalamus (VMH) is fundamental to the hormonal regulation of sociosexual behaviors in the female Syrian hamster. Nonetheless, little is known about the organization or connectivity of the VMH in the hamster. In the coronal plane, we find the VMH to be roughly spherical in shape, with most of the nucleus staining in a homogeneous pattern. In the rostral half of the nucleus there is a medial subdivision seen as a compact cluster of cells, and a lateral subdivision that is crescent-shaped with a long dorsal-ventral axis.

Efferent projections of VMH neurons were mapped following iontophoretic injections of the plant lectin, Phaseolis vulgaris leucoagglutinin. VMH efferents in the Syrian hamster are similar to those reported for the rat, with primary forebrain projections to the lateral septum, bed nucleus of the stria terminalis, medial preoptic area, anterior hypothalamus, several amygdaloid nuclei, and the paraventricular thalamus. Efferent projections to the midbrain include the central gray and several tegmental fields. Unlike the rat, innervation of the pretectum and deep layers of the rostral superior colliculus is also apparent. We are currently studying possible differences in efferent connectivity of the subdivisions of the VMH. Supported by NIH grant HD21478.

471.3

LOCALIZATION OF TYROSINE HYDROXYLASE IMMUNOREACTIVE (TH-ir) NEURONS IN THE HYPOTHALAMUS OF CATS AND RHESUS MONKEYS.

A. Jayaraman, H. Hu* and J.K. Rao*. Depts of Neurology and Pediatrics, Louisiana State Univ. Sch. of Med, New Orleans, La. 70112.

Immunocytochemical studies suggest that the distribution pattern of TH-ir neurons in the hypothalamus may vary in different species of animals and even within the same strains of mice. The organization of catecholaminergic neurons in the monkey hypothalamus has not been studied in detail. Our study of the distribution pattern of TH-ir neurons in cats and monkeys with the avidin-biotin immunohistochemical method showed numerous TH-ir neurons in the paraventricular nucleus and in zona incerta of the dorsal hypothalamus; in the periventricular nucleus and the posterior hypothalamic areas of the intermediate regions of the hypothalamus. In the ventral hypothalamus TH-ir cells were clustered in the infundibular nucleus. These patterns of localization of hypothalamic catecholaminergic neurons were similar to those described in cats and monkeys using fluorescence histochemical methods. In addition to these regions, TH-ir neurons were also noted prominently in the preoptic, supraoptic, and the dorsomedial hypothalamic areas. These areas were not recognized in earlier studies to contain catecholaminergic cells. The distribution pattern of TH-ir cells in hypothalamus of cats and monkeys show many similarities to the pattern described in mouse using immunohistochemical methods (Ruggiero et al., JCN.223:556,1984). The TH-ir neurons in the preoptic hypothalamus (along with their interactions with neurotensin) may provide the anatomical substrate for the thermoregulatory role of dopamine. Supported by the Scottish Rite program for Schizophrenia Research.

471.4

MONOAMINERGIC INNERVATION OF PEPTIDERGIC NEURONS IN THE RAT HYPOTHALAMUS: A DOUBLE IMMUNOENZYMATIC STAINING.

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The monoaminergic (MA) innervation of magnocellular hypothalamic neurons was studied with a double immunocytochemical technique using two different substrates for peroxidase:diaminobenzidine and benzidine dihydrochloride. The greater input to vasopressinergic (VP) cells was by far the noradrenergic (NA) one, with fibers surrounding the VP cell bodies. In the contrary, only a few dopaminergic (DA) varicosities contacted VP perikarya, most often in the more anterior parts of the supraoptic (SON) and paraventricular (PVN) nuclei. The serotonergic (5HT) innervation appeared to contact subpopulations of VP cells, both in the SON and in the peripheral parts of the posterior magnocellular PVN. None of these three transmitters innervated the whole oxytocinergic (OX) population. They appeared to be directed toward groups of OX neurons. The NA and 5HT fibers contacted a greater number of OX perikarya than did the DA ones in both nuclei.
Except for the NA afferents on VP neurons, each type of MA fibers seems to innervate only certain subpopulations of VP or OX cell bodies. It remains to be determined if the information carried by these three sets of afferents are sent to separate groups of magnocellular peptidergic neurons, or if a same cell can integrate the different synaptic events of these MA systems.

471.5

FUNCTIONAL AND MORPHOLOGICAL STUDIES ON PUTATIVE INTERACTIONS BETWEEN NEUROPEPTIDE Y AND LUTEINIZING HORMONE RELEASING HORMONE IN RATS AND MONKEYS.

C.W. Coen[1], M.-C. Ruiz de Elvira[1]*, K. Maclachlan[1]*, C. Montagnese[1]*, M.J.D. Andrews[1]*, K.T. O'Byrne[2]*, I.J. Clarke[3]* and Zs. Liposits[4]*. [1]Department of Anatomy & Human Biology, King's College London, UK; [2]MRC Reproductive Biology Unit, Edinburgh, UK; [3]Prince Henry's Hospital, Melbourne, Australia; [4]Dept. Anatomy, Pecs, Hungary.

Ovariectomized rats treated with oestrogen and progesterone show an increased release of luteinizing hormone (LH) following intraventricular (i.c.v.) neuropeptide Y (NPY; 1 ug). Prior treatment (-10 min i.p.) with the α_2-adrenergic antagonists piperoxane (50 mg/kg) or idazoxone (2 mg/kg) prevents this stimulation. In contrast, the pulsatile release of LH occurring in ovariectomized rats which have not been treated with steroids is suppressed by i.c.v. NPY (1 ug) and this effect is unaffected by piperoxane. NPY may also be involved in the hypothalamic control of LH release in primates since 1 ug given i.c.v. to oestrogen-treated ovariectomized marmosets results in a stimulation of LH comparable to that induced by i.v. administration of 100 ng LH releasing hormone (LHRH). Immunohistochemical studies on adjacent sections from the rat, marmoset or macaque show a strong correspondence between LHRH perikarya and presumptive terminal fields containing NPY. With double-label immmohistochemistry (using the conventional DAB reaction product together with the nickel- or silver-intensified version in the same section) we have found NPY varicosities in putative approximation to LHRH perikarya in each of the three species studied. Confirmation of a synaptic relationship underlying the functional effects awaits completion of studies at the EM level.

471.6

MEDULLARY PROJECTIONS TO THE LUTEINIZING HORMONE RELEASING HORMONE FORMATION OF THE RAT: STUDIES INVOLVING RETROGRADE TRACING AND IMMUNOHISTOCHEMISTRY FOR NEUROPEPETIDE Y AND PHENYLETHANOLAMINE N-METHYLTRANSFERASE.

A. Castaneyra-Perdomo*, M. Bennett* and C.W. Coen (SPON: N.H.K. Holder). Dept. Anatomy & Human Biology, King's College London, U.K.

Retrograde tracing and immunohistochemical techniques have been used to identify cells in the medulla oblongata which project to the region of the luteinizing hormone releasing hormone (LHRH) perikarya in the preoptic area of the rat hypothalamus. Wheatgerm agglutinin-horseradish peroxidase was injected into the LHRH formation by iontophoresis. Three days later the rats were treated with i.c.v. colchicine (105 ug) and on the following day they were perfused transcardially with 5% acrolein. Sections through the preoptic area were reacted using the conventional DAB method to reveal the injection site. These sections were then processed for LHRH immunoreactivity using the nickel-intensified reaction. Thus, success in the placement of the micropipette was indicated by the presence of the dark blue LHRH perikarya within the brown injection site. Sections through the medulla were reacted for the retrogradely transported lysosomal peroxidase using the nickel-intensified method and these sections were subsequently processed for immunoreactivity for neuropeptide Y (NPY) or the adrenaline synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT) using the DAB method. Many cells within the areas corresponding to the ventrolateral and dorsomedial adrenaline/NPY cell groups (C1 and C2) were found to contain the retrograde tracer; a minority of the cells also contained NPY- or PNMT-immunoreactivity as revealed by dark blue lysosomes within a brown perikaryon.

471.7

PROJECTIONS FROM THE TUBEROMAMMILLARY NUCLEUS OF THE HYPOTHALAMUS TO PRIMARY AFFERENT NEURONS IN THE MESENCEPHALIC NUCLEUS OF THE TRIGEMINAL NERVE.

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The somas of primary afferent neurons in the mesencephalic nucleus of the trigeminal nerve (Mes V) in rat are surrounded by a dense plexus of adenosine deaminase-immunoreactive (ADA-IR) axons which originate, in part, from ADA-IR neurons located in the tuberomammillary nucleus (TM) [Nagy et al., (1986) Neuroscience 17, 141-156]. Projections of TM to Mes V were investigated by ultrastructural, anterograde tracing and immunohistochemical techniques. After injections of Phaseolus vulgaris-leucoagglutinin (PHA-L) into the region of TM, PHA-L-labelled ADA-IR fibers were seen around Mes V neurons. Ultrastructurally, ADA-IR axons were varicose and contained ADA-IR vesicles 45 to 70 nm in diameter. Occasionally, thin axons could be traced back to local ADA-positive neurons. Synaptic junctions were sometimes seen between Mes V somas and ADA-IR terminals. It is suggested that the hypothalamus, via a direct action on Mes V neurons which convey proprioceptive input from jaw muscles, may exert autonomic control over jaw movements related to aggressive attack, defensive or feeding behavior.

471.8

POTENTIAL TARGET NEURONS OF A SEXUALLY DIMORPHIC ENKEPHALINERGIC FIBER SYSTEM IN THE PREOPTIC AREA.

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A sexually dimorphic enkephalinergic fiber system, in which expression is governed by the gonadal steroids in both an organizational and activational manner, is present in the periventricular preoptic nucleus (pePOA) of the rat in only the female. In previous electron microscopic studies, we have identified abundant enkephalinergic terminals and synapses in the pePOA. Thus, in an effort to determine its functional significance, it is important to identify the target neurons of this system. Colchicine treated and untreated female Sprague-Dawley rats were perfused and sections were processed for the immunocytochemical localization of met-enkephalin (m-ENK), neurotensin (NT), substance P (SP), cholecystokinin (CCK), somatostatin (SS), corticotropin releasing factor (CRF), luteinizing hormone releasing hormone (LHRH), vasoactive intestinal polypeptide (VIP), oxytocin (OXY), vasopressin (VP), and tyrosine hydroxylase (TH). No LHRH, VIP, OXY, VP, or CRF cells were detected in the pePOA. Only widely scattered m-ENK, NT, SP, and SS cells were present, while greater numbers of these cells were detected immediately lateral or posterior to it. A comparatively higher density of CCK positive cells was present in the pePOA at the level of the sexually dimorphic m-ENK system. However, much greater numbers of these cells were present at a posterior position in the pePOA, immediately caudal to the region where the m-ENK system is maximally expressed. A relatively large number of TH positive cells are present in the pePOA at the level of the dense m-ENK fiber plexus. Double labelling studies using nickel-enhanced DAB (for m-ENK fibers) in conjuction with DAB (for TH immunoreactivity) indicate close apposition between m-ENK varicosities and TH positive cells, suggesting potential synaptic interactions between these systems. {Supported by NS-23591}

471.9 WITHDRAWN

472.1

CHARACTERISTICS OF PARASYMPATHETIC SECRETOMOTOR NEURONS STUDIED IN VITRO. T.A. Harrison and R.M. Bradley. Sch. Dent., Univ. Michigan, Ann Arbor, MI 48109.

We are investigating the secretomotor control of von Ebner's lingual salivary glands in the rat. These glands secrete directly into the clefts of the circumvallate and foliate papillae and provide the microenvironment for lingual taste buds contained in the epithelium lining these clefts. The secretomotor cells controlling von Ebner's glands are closely associated with the second order gustatory relay nucleus in the brainstem (solitary nucleus), suggesting interactions between gustatory afferent information and secretomotor activity. Because it is difficult to record from the parasympathetic cells in vivo, we have developed a brain slice preparation of the medulla that contains both the gustatory and parasympathetic nuclei. Horizontal brain slices were prepared in a conventional manner and cut at a 20° angle. Such slices included not only the entire solitary nucleus but also the stump of the glossopharyngeal nerve, which can be electrically stimulated to activate both the solitary tract and the secretomotor cells. Using this preparation we have successfully recorded spontaneous activity from neurons in the caudal and rostral solitary nucleus for up to 5 hours. Rhythmically active neurons were recorded in the caudal solitary nucleus whereas neurons in the gustatory, rostral solitary nucleus showed a highly irregular pattern of activity. Successful stimulation of the solitary tract to activate solitary nucleus neurons has been accomplished using a bipolar stimulating electrode placed on the glossopharyngeal nerve stump. (Supported by NIH Grant NS21764.)

472.2

ADENOSINE INVOLVEMENT IN A LONG-LASTING BRAINSTEM-MEDIATED INHIBITION OF PHRENIC ACTIVITY FOLLOWING HYPOXIA. E.A. Gallman and D.E. Millhorn. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, North Carolina 27599.

Among the long-lasting responses of central respiratory control network is an inhibition of phrenic nerve output, evoked by brief exposure to hypoxia, which lasts at least one hour and requires the presence of the midbrain (J. Physiol. 395:333, 1988). These studies were done to investigate the neurochemical basis for this inhibition. All studies were performed in cats which were anaesthetized, peripherally chemo-denervated, paralyzed, and artificially ventilated with 100% O_2. End-tidal PCO_2 and temperature were servo-controlled. Central respiratory drive was determined as the product of peak integrated phrenic nerve activity and phrenic rate. Cats were decerebrated above the midbrain and then exposed to hypoxia (F_IO_2 = .10 to .15) for 10 min to elicit long-lasting phrenic inhibition. Theophylline, an adenosine receptor antagonist, was then introduced (13.6 mg/kg i.a + 13.6 mg/kg/hr i.v.) and exposure to hypoxia repeated. In the presence of theophylline, the phrenic inhibition following hypoxia was prevented. In another series of cats, adenosine, pressure injected into the nucleus tractus solitarus (NTS) of the medulla elicited inhibition of phrenic activity. These results implicate adenosine in the long-lasting post-hypoxic inhibition of phrenic activity. This work was supported by USPHS Grant HL-33831.

472.3

CHOLINESTERASE-NEGATIVE VAGAL EFFERENTS PROJECTING TO THE ABDOMEN IN THE RAT. E.K.Tayo* and R.G.Williams* (SPON: G.J.Dockray). Dept. Phys.Pharm., The Univ., Southampton, SO9 3TU, UK.

Vagal parasympathetic preganglionic neurones innervating the abdominal viscera, arise from the Dorsal Motor Nucleus of the Vagus (DMN). These neurones are generally regarded as cholinergic, but a number of other transmitter candidates have been suggested.

As a first step towards assessing the relative proportions of different neurochemical types of vagal efferents present, we have examined the distribution of cholinesterase positive cells in the DMN of the rat.

Efferents projecting to the abdomen were identified by retrograde tracing of True Blue (TB) from the stomach wall, and the proportions containing acetylcholinesterase (AChE) assessed.

The distribution of TB cells was similar to that of the AChE cells and reflected the general shape of the DMN. However, only a proportion, less than 40%, of the TB cells were AChE positive.

CONCLUSION: It follows that, either the majority of abdominal vagal efferents are non-cholinergic or that at least two populations of cholinergic cells exist within the DMN.

472.4

EVIDENCE FOR A SOMATOSTATINERGIC PROJECTION FROM THE NUCLEUS OF THE SOLITARY TRACT TO THE NUCLEUS AMBIGUUS IN THE RAT. E.T. Cunningham, Jr.,[1,2] R. Benoit,[3] and P.E. Sawchenko.[2] The Univ. of Calif.[1] and The Salk Institute[2], La Jolla, CA. and Mont. Gen. Hosp.[3], Montreal, Canada.

Axonal transport and immunocytochemical methods were used to investigate the anatomical and biochemical organization of projections from the nucleus of the solitary tract (NTS) to the compact, esophageal part (see Beiger and Hopkins, JCN 262:546, '87) of the nucleus ambiguus (NA) in the rat. Iontophoretic deposits of the retrograde tracer fluorogold (FG) were placed in the compact NA. These deposits labeled a column of cells just medial to the solitary tract and rostral to the obex, which we term the central part of NTS (after Ross, et. al., JCN 242:511, '85). Iontophoretic deposits of the anterograde tracer Phaseolus vulgaris-leucoagglutinin (PHA-L) placed in the central part of NTS gave rise to dense and topographically restricted projections to the compact NA. More caudal and ventral aspects of the NA did not receive a prominent input from the central part of NTS, and deposits that spared the central part of NTS gave rise to only sparse projections to the compact NA. Antisera against somatostatin-28 (SS-28) revealed immunoreactive cell bodies within the central part of NTS. In addition, an immunocytochemical double-labeling procedure, capable of co-localizing both PHA-L and endogenous peptides to single fibers and/or varicosities, demonstrated an appreciable number of SS-28-immunoreactive terminals within the compact NA that arose from the NTS. These data provide evidence for a discrete somatostatinergic projection from the central part of NTS to the compact NA. Studies implicating the central part of NTS and the compact NA in esophageal function suggest that this pathway may be involved in the reflexive control of esophageal motility.

472.5

CONTRIBUTION OF THE ROSTRAL VENTROLATERAL (RVLM) AND ROSTRAL VENTROMEDIAL (RVMM) MEDULLA TO MAINTENANCE OF SYMPATHETIC NERVE ACTIVITY (SNA). K.J. Varner, C.L. Grosskreutz* and M.J. Brody. Dept. of Pharmacol. and CV Center, University of Iowa, Iowa City, IA 52242

Studies from this laboratory showed that rostral ventral medulla (RVM) contains distinct subregions which differentially regulate vasomotor function. The purposes of this study were to determine 1) the contribution of these subregions to maintenance of sympathetic tone and 2) whether RVLM (and/or RVMM) is the sole source of sympathetic outflow. Rats were prepared for measurement of heart rate, arterial pressure (AP) and renal SNA and were artificially ventilated. Reversible inactivation of RVLM (2 mm) and RVMM (1mm) lateral to midline was achieved by bilateral microinjection of lidocaine (LIDO) (200 nl, 4%). Residual SNA and neurogenic cardiovascular tone after LIDO into RVLM and RVMM was estimated by iv administration of trimethaphan (TRIM). Administration of LIDO into RVMM decreased SNA 39±10% and AP 51±12 mmHg to 59±5 mmHg. Administration of LIDO into RVLM decreased SNA 41±15% and AP 51±11 mmHg to 62±3 mmHg. After LIDO into all four sites (RVLM + RVMM) SNA fell 56±10% and AP fell 58±6 mmHg to 51±4 mmHg. TRIM produced additional falls in SNA of 41±10 % and AP of 8±3 mmHg. We conclude that 1) RVMM and RVLM contribute equally to SNA and vasomotor tone and 2) a substantial fraction of SNA appears to be generated outside of RVM but has a limited role in vasomotor control. (Supported by HLB-14388 and Berlex Labs.)

472.6

EFFERENT AND AFFERENT CONNECTIONS OF THE ROSTRAL VENTROLATERAL MEDULLA OF THE RABBIT. D.R. Liskowsky, P.L Vera, R.W. Winters*, P.M. McCabe, C.G. Markgraf, N.Schneiderman. Dept. of Psychology, Univ. of Miami, Coral Gables, FL. 33124.

The rostral ventrolateral medulla (RVLM), which encompasses the C1 noradrenergic cell group, is known to play an important role in cardiovascular regulation. Activation of this region in the rabbit, with either electrical or chemical stimulation, produces a profound pressor response, usually accompanied by bradycardia. It is thought that this region is essential to the mediation of the pressor component of the 'defence' response elicited by electrical stimulation of the hypothalamus and midbrain.

Using the retrograde fluorescent tracer Fluorogold (FG), and the retrograde and anterograde tracer horseradish peroxidase conjugated with wheat germ agglutinin (HRP) both the afferent and efferent connections of the RVLM in the rabbit were mapped. In New Zealand albino rabbits either FG or HRP (20-50 nl) were injected using glass micropipettes (tip o.d. 20-40μ) connected to a micro-pressure injection system. To facilitate placement of the tracers, pulsed electrical current (25-75μA) was passed through the pipette to elicit a pressor response from RVLM.

Injection of FG or HRP into the RVLM led to cell body labelling in several mesencephalic and diencephalic structures, including the periaqueductal grey region of the midbrain, the parabrachial nucleus, and the paraventricular nucleus and perifornical region of the hypothalamus. Retrograde labelling was also seen in the dorsal medial medulla, including the nucleus tractus solitarius. HRP histochemistry also revealed anterograde labelling in many of the same structures which showed retrograde labelling, thereby suggesting reciprocal fucntional connections. Anterograde labelling of HRP was also observed in the intermediolateral cell column of the spinal cord. Supported by NIH Grants HL07426 and HL 36588.

472.7

AN ANTEROGRADE AND RETROGRADE ANATOMICAL ANALYSIS OF RETICULO-RETICULAR CONNECTIONS IN THE RAT. R.P. Vertes. Mercer University, School of Medicine, Macon, GA 31207.

In previous analyses of ascending reticular formation (RF) projections in the rat using tritiated amino acids (Vertes et al., Neuroscience 19:873, 1986; Vertes and Martin, JCN, in press), we described pronounced RF projections to adjacent reticular fields. It was difficult, however, to unequivocally determine that ascending RF fibers terminated in, rather than coursed through, rostral levels of the RF. The present study was designed to answer this question and to determine the major inputs to the medullary (mRF), pontine (PRF) and midbrain (MRF) reticular formation.

WGA-HRP retrograde and PHA-L anterograde techniques were used. The major findings were as follows: (1) the major inputs to all levels of the brainstem RF were from other regions of the RF; (2) the PRF and mRF received pronounced projections from the contralateral PRF and mRF, respectively, but the MRF received relatively few contralateral MRF projections; (3) each level of the RF received significantly more projections from adjacent caudal as opposed to rostral levels of the RF such that nucleus gigantocellularis (NGC) received major inputs from nuclei reticularis dorsalis and ventralis of the lower medulla, nuclei pontis caudalis and oralis from NGC, and the MRF from the PRF; (4) both PRF and MRF received very few projections from lateral parvocellular regions of the RF; the mRF received moderate to heavy parvocellular projections; and (5) the PRF and mRf received heavy projections from an area of the MRF that is co-extensive with mesencephalic locomotor region. The results indicate extremely pronounced reticulo-reticular interconnections and suggest a hierarchical arrangement such that each level sends its major projection to the next rostral level.

472.8

RESPONSES OF NEURONS IN THE PONTINE SWALLOWING NUCLEUS OF LAMBS TO MULTIMODAL STIMULATION OF THE ORAL CAVITY AND LARYNX. R.D. Sweazey and R.M. Bradley. Sch. Dent., Univ. Michigan, Ann Arbor, MI 48109.

We have investigated the responses of neurons in a region of the lamb pons termed the pontine swallowing nucleus. Neurons were characterized by their responses to oral cavity and laryngeal stimulation with chemical, mechanical and thermal stimuli, by response latency, and receptive field location and size. Responsive neurons were found medial to the dorsomedial tip of the sensory trigeminal nucleus. These pontine neurons had a high spontaneous rate and over 50% responded exclusively to only one stimulus modality. The order of effective stimuli was mechanical, chemical, thermal. The remaining neurons showed multimodal responses, with the majority of these cells responding to both chemical and thermal stimuli. Responses to mechanical stimuli were observed with moving and stationary stimuli, the moving stimulus being more effective. Chemosensitive neurons in the lamb pons had responses similar to those previously observed in the nucleus of the solitary tract. Responses to thermal stimuli were observed more frequently to cooling than to warming. The receptive fields of neurons which responded to mechanical and/or thermal stimuli were larger than the receptive fields of neurons which responded to chemical stimuli. Furthermore, neurons responsive to mechanical stimuli had shorter response latencies than those responsive to chemical stimuli. Analysis of response latencies suggests that these pontine neurons receive direct afferent input from the oral cavity and larynx. (Supported by NIH grant DE05727.)

472.9

AFFERENT PROJECTIONS TO THE MEDIAN RAPHE NUCLEUS IN THE RAT. Joseph M. Paris and Stanley A. Lorens. Behavioral Pharmacol. Lab., Dept. Pharmacol., Loyola Univ. Med. Cnt., Maywood, IL 60153 USA.

The efferent projections of the midbrain raphe nuclei in the rat have been studied by many investigators. However, the afferent projections to these nuclei, in particular, the median raphe nucleus (MR) have not been thoroughly examined. Male Sprague-Dawley rats received an iontophoretic application of the fluorescent retrograde dye, Fluoro-Gold^{R}, into the MR. The rats were anesthetized and transcardially perfused 7-14 days post-injection. Coronal (18-24 μm) sections were obtained at 0.1 mm intervals throughout the brain and spinal cord.

Several regions of the neuroaxis project to the MR. In terms of the number of retrogradely labelled cell bodies, the major sources of afferent fibers are: medial frontal cortex, vertical limb of the diagonal band of Broca, lateral habenula, lateral hypothalamus, interpeduncular nucleus, reticular formation, and the lateral dorsal tegmental nucleus. Other sources of afferent fibers include: lateral septum, zona incerta, ventral tegmental area, central grey, dorsal raphe nucleus, caudal raphe nuclei, medial nucleus of the cerebellum, and the spinal cord. Thus the activities of both the serotonergic and non-serotonergic cell bodies in the MR can be modulated by fibers which originate in several distinct regions of the central nervous system.

472.10

PROJECTIONS FROM THE RAPHE ONTO MEDULLARY CATECHOLAMINERGIC NEURONS. A.P. Nicholas* and M.B. Hancock. Department of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, Texas 77550.

Phaseolus vulgaris-leucoagglutinin (PHA-L) was iontophoretically deposited into the nucleus raphe magnus, nucleus raphe obscurus and nucleus reticularis gigantocellularis pars alpha of anesthetized Sprague-Dawley rats. Following survival times of 3-5 days, the rats were anesthetized and perfused with 4% paraformaldehyde. The medulla was removed and sectioned (30 μm) with a Vibratome. PHA-L-immunoreactive (PHA-LI) elements were immunostained black with the PAP technique and nickel-intensified diaminobenzidine (Ni/DAB), while phenylethanolamine N-methyl transferase-immunoreactive (PNMTI) neurons or tyrosine hydroxylase-immunoreactive (THI) neurons were immunostained amber with the PAP technique and DAB alone. Black-stained PHA-LI cells were present at the deposition sites in the raphe and PHA-LI terminal fields were present among amber-stained PNMTI cells in the rostral medulla or THI cells in the caudal medulla. Varicose PHA-LI fibers were observed coursing along the surface of PNMTI and THI dendrites, and PHA-LI boutons were contiguous with somata of PNMTI and THI cells. These results indicate that medullary adrenergic and noradrenergic neurons are influenced by projections from the medullary raphe nuclei. These raphe projections are a likely source for 5HT-immunoreactive endings that have been shown to be contiguous with medullary catecholamine cells. Supported by The American Heart Assoc., Texas Affiliate.

472.11

ENHANCEMENT OF THE PERFORANT PATH POPULATION SPIKE BY STIMULATION OF LOCUS COERULEUS: EVIDENCE FOR TWO SEPARATE MECHANISMS. C.W.Harley, J.S. Milway* and S.Evans*. Dept of Psychology, MUN, St. John's, Nfld. A1B 3X9

Electrical stimulation in the vicinity of locus coeruleus (LC) has been shown to produce reliable and significant enhancement of the perforant path (PP) evoked population spike in the dentate gyrus of urethane-anesthetized rats. Microejection of glutamate in or near LC also induces significant enhancement of the PP evoked population spike. Both forms of enhancement appear similar in that the main effect is on population spike amplitude and enhancement can be long-lasting. We had assumed that both forms of enhancement were mediated by the release of norepinephrine (NE) in the dentate gyrus since NE can induce both short and long-lasting enhancement of the PP evoked population spike when applied to the hippocampus in vivo or in vitro. These effects of NE depend on activation of a beta receptor. In agreement with this assumption we were able to block the glutamate-induced enhancement of the population spike by administering propranolol. We were not able to block the enhancement effect produced by electrical stimulation of LC. In some experiments both glutamatergic and electrical LC activation were produced by a convergent pipette and electrode assembly. Propranolol blocked the enhancement produced by glutamate stimulation but not that produced by electrical activation.

These results suggest there are two systems in the vicinity of LC capable of producing both short- and long-lasting enhancement of the PP evoked population spike. One of the systems appears to be the LC whose effects are mediated by beta receptors in the hippocampus. The other system has not been identified pharmacologically and it is unlikely that the cell bodies of this system occur in the vicinity of LC.

472.12

EFFECTS OF STIMULUS INTENSITY AND REPETITION RATE ON BRAIN STEM AUDITORY EVOKED POTENTIALS (BAEPs) IN AWAKE MONKEY. J. A. Pineda[1], T. C. Holmes[1*], D. Swick[2*], and S. L. Foote[1,3]. Depts. of Psychiatry[1] and Neuroscience[2], UCSD, and Scripps Clinic and Research Foundation[3], La Jolla, CA 92093.

While an extensive database of knowledge exists concerning human BAEPs, there is a limited amount of non-human primate data, especially from unanesthetized animals. In this study, BAEPs were recorded in nine unanesthetized squirrel monkeys (Saimiri sciureus) from a chronically implanted epidural electrode in vertex, referenced to an inion electrode. The effects of 30, 50, 60, and 70 dB SPL clicks or repetition rates of 5, 10, 20, 40, and 80 Hz were analyzed in different sessions. Monkey waveforms resembled human BAEPs in morphology, number of components, polarity, latency variability, and inter-peak conduction times. They consisted of seven components (I-VII), six exhibiting positive polarity, some exhibiting multiple peaks, and all exhibiting similar latencies in different sessions. Additionally, slow potentials (SN, SP) recorded during the second half of the epoch provided a substratum for waves IV through VII. Systematic decreases in latency with increasing intensity were significant for most components, while increases in repetition rate increased latencies only in some components. Inter-peak conduction times resembled human data while differing from those reported for anesthetized monkeys. These similarities in conduction latencies suggest that times required for impulses to reach different brain stem areas involved in acoustical processing may be conserved across species and sizes of brain. Thus, given the similarities to human potentials, BAEPs in awake monkey may be valuable for investigations into the origins of these components.

472.13

BRAIN STEM INHIBITION IN ADAPTATION

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A vestibular model of adaptation is utilized to test the hypothesis that inhibitory processes in the brain stem determine the extent and rapidity of adaptation to a changing environment. Brain stem inhibition is initiated by a vagal afferent mechanism which is activated by electrical stimulation of specific nerve groups. Four conscious and unrestrained adult male and female squirrel monkeys of Bolivian origin were exposed to 30 rpm horizontal rotation in a transparent plastic test chamber for up to 2 hours/day for 6 to 12 days. Rotation was sustained for the entire session lasting from 15 to 120 minutes with multiple vomiting episodes permitted. Vomiting latency and frequency were measured in both stimulated and non-stimulated animals. Implantation of a vagal cuff is performed on monkeys anesthetized with sodium pentobarbital IV (35 mg/kg). A pair of adjacent contacts is used for bipolar stimulation at 1-10 ma amplitude, 4-100 Hz frequency and 0.3-0.6 msec. duration. Electrode connections are made with contacts housed in a nylon receptable cemented to the top of the skull. Each monkey had a substantial reduction in emetic response to rotation during vagal activation. The percentage reduction in each of the four animals was 81% (#1), 88% (#2), 72% (#3) and 83% (#4). The average percent reduction for all animals was 81%. Thus, adaptation can be enhanced by a vagal afferent mechanism incorporating inhibitory transmitters.

472.14

PATHWAYS DESCENDING FROM SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA SHOWN BY PHASEOLUS VULGARIS LEUCOAGGLUTIN. M.R. Park. Dept. Anatomy & Neurobiology, Univ. Tennessee, Memphis, The Health Science Center, Memphis, TN 38163.

Injections of the anterograde tracer phaseolus vulgaris leucoagglutinin (PHAL) in substantia nigra (SN) or ventral tegmental area (VTA) produces labeling of extensive descending pathways. Injections in VTA can fill the entire mesencephalon, as seen in coronal section, with anterogradely labeled varicose axons and terminal fields. In sagittal section, terminal fields can be seen throughout the midline raphe nuclei and as far caudal as the pontine reticular nuclei. A more discrete distribution to the forebrain projecting raphe nuclei, dorsal and median raphe, as well as to raphe pontis, is seen when the injection site is in lateral VTA and medial SN. Anterogradely labeled axons are in the order of 0.5 μm in diameter. Few descending projections are seen in tegmentum from SN pars reticulata injections although ipsilateral superior colliculus is extensively labeled with axons arranged in a pattern of pillars and laminae that surround blank holes.

The extent of labeling from SN and VTA is greater than that reported for dopaminergic connections, implying that a substantial portion of these descending axons are non-dopaminergic. It should be born in mind, however, that these injections have been made in areas that carry heavy descending traffic and so test to the fullest extent possible the assertion that PHAL does not label fibers of passage.

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472.15

IMMUNOHISTOCHEMICAL LOCALIZATION OF GABA IN THE RAT INTERPEDUNCULAR NUCLEUS (IP). J.C. Pearson. Dept. of Anatomy, Wright State Univ. Sch. of Med., Dayton, OH 45435.

Evidence for glutamic acid decarboxylase and GABA receptors within rat IP suggests a neurotransmitter role for GABA in this nuclear complex. In the present study, antiserum against GABA-glutaraldehyde-keyhole limpet hemocyanin was used to localize GABA-like immunoreactivity (GABA-LI) in rat IP. Rats were colchicinized and perfusion fixed (4% paraformaldehyde, 0.5% glutaraldehyde, 0.5% $K_2Cr_2O_7$ in 0.05M PO_4 buffer, pH 6.5). Vibratome sections (50μm) were processed by the avidin-biotin-peroxidase method. Staining was blocked by 100μM GABA-glutaraldehyde conjugate but not by 500μM conjugated glutamate, glutamine, or taurine. GABA-LI neuronal somata were abundant in rostral (R), dorsomedial (DM), central (C), intermediate (I), and dorsal lateral (DL) subdivisions of IP, but, found infrequently in the rostral lateral (RL) and lateral (L) nuclei and absent from the apical (A) subnucleus. GABA-LI processes were present in all IP subnuclei but prominent in IP-RL, -L and -A. Quantitative evaluation of soma cross-sectional areas indicated that GABA-LI somata were among the largest of all neurons in IP-DM and -R, but among the smallest of cells in the remaining subnuclei. Results are consistent with the hypothesis that GABA has a neurotransmitter role in IP function, and suggest that the influence of IP GABA-LI cells may be extrinsic as well as intrinsic to this nuclear complex.

472.16

Mesopontine Tegmental Projections to the Nucleus Basalis of Meynert: An Ultrastructural Study. A.E. Hallanger, S.D. Price*, T. Steininger, and B.H. Wainer. The University of Chicago, Committee on Neurobiology. Chicago, IL 60637.

The existence of a cholinergic projection from the pedunculopontine tegmental nucleus (PPT) in the midbrain to the cholinergic nucleus basalis of Meynert in the forebrain has been suggested (Woolf and Butcher, '86; Semba et al., '88). In order to determine whether the cholinergic neurons of the nucleus basalis receive input from a cholinergic source such as the PPTor the laterodorsal tegmental nucleus (LDT), or from nocholinergic neurons in the mesopontine tegmentum, we used rats treated with the following combinations of techniques: (1) ChAT immunocytochemistry only, (2) anterograde transport of PHA-L from the region of PPT to label midbrain afferent terminals in the forebrain, combined with ChAT immunocytochemistry to label cholinergic nucleus basalis neurons, and (3) anterograde transport of PHA-L from the region of the PPT, with retrograde transport of WGA-HRP from the cerebral cortex to label cortically-projecting nucleus basalis neurons. Tissue from all types of cases was examined in the electron microscope. We found that: (1) Cholinergic somata and dendrites (both distal and proximal) in the nucleus basalis of Meynert did not receive synaptic input from ChAT-immunoreactive synaptic terminals. However, numerous non-ChAT-immunoreactive terminals did contact cholinergic dendrites, and cholinergic terminals did contact noncholinergic dendrites; (2) Axons labelled with PHA-L from the mesopontine tegmentum did not form synaptic contacts onto cholinergic somata or dendrites in the basal forebrain, but did contact noncholinergic neurons in this region; and (3) PHA-L labelled axons did not form synaptic contacts onto somata retrogradely labelled from the cerebral cortex, but did synapse onto non-retrogradely labelled neurons in the basal forebrain. These results suggest that there is little, if any, cholinergic input from the PPT or LDT onto the cholinergic basal forebrain neurons. Supported by PHS HD-04583, PHS NS-17661(BHW) and PHS 5T32 GM-07281(AEH).

473.1

CONDITIONED TASTE AVERSIONS AFFECT GUSTATORY EVOKED ACTIVITY IN THE NTS OF CHRONIC DECEREBRATE RATS. G. P. Mark and T. R. Scott. Dept. Psychol., Princeton Univ., Princeton, NJ 08544, and Dept. Psychol. & Inst. Neurosci., Univ. Delaware, Newark, DE 19716

Decerebrate rats exhibit satiety and normal oro-facial reactions to tastants, but do not respond to the challenges of dehydration or sodium depletion. Nor do they exhibit the ability to acquire or maintain conditioned taste aversions (CTA's). To explore the possibility that taste cell activity is modified by CTA learning in these preparations, responses were recorded from 42 single neurons in the nucleus tractus solitarius (NTS) of chronic decerebrate rats in which ingestion of 2.5 mM saccharin (CS) was paired with LiCl-induced malaise. Results were compared with those reported previously for unconditioned decerebrates (Mark et al., 1988) and also to data from conditioned intact rats (Chang & Scott, 1984). When neurons were categorized into sweet and non-sweet responsive groups, responses evoked by the CS from sweet-sensitive cells were shown to be increased by 16%. Post-stimulus time histograms revealed that this increase was due largely to a spike of activity, three times greater than unconditioned decerebrate levels, which peaked 950 msec after stimulus onset. Multidimensional scaling analyses, however, did not reveal a shift in the coding of the CS following conditioning. Whereas stimulus profiles evoked by the CS and quinine became more similar with CTA development in intact animals, these profiles remained distinct in decerebrates. These results demonstrate that a subset of NTS neurons are functionally altered as a result of CTA learning independent of forebrain influences. The absence of a change in coding of the CS in decerebrates, however, may account for the fact that these animals do not demonstrate CTA learning behaviorally.

473.2

CHRONIC RECORDING IN AND AROUND THE HYPOGLOSSAL NUCLEUS DURING INGESTION AND REJECTION OF SAPID STIMULI IN THE RAT. J.B. Travers and L.M. Jackson*. Dept. Oral Biology, Ohio State Univ., Columbus, OH 43210

The neural substrate for ingestion and rejection is organized in the caudal brain stem (Grill & Norgren, '78). Gustatory stimuli influence the motoneurons producing ingestion and rejection primarily over polysynaptic pathways (Travers & Norgren, '83). We have begun to examine the neural circuitry underlying these responses by examining gustatory elicited responses of ingestion and rejection in single cells of the hypoglossal nucleus and the adjacent reticular formation. Under Nembutal anesthesia, rats were implanted with fine wires in the anterior digastric (AD), styloglossus (STY) and thyropharyngeus (PHA) muscles. In addition, a chronic microdrive was positioned over the hypoglossal nucleus and secured to the cranium. Wires were brought to an Amphenol connector for subsequent attachment to the instrumentation. Intra-oral cannulas were also implanted for the delivery of gustatory stimuli. Rats were tested in a Plexiglass observation chamber. Unit activity was recorded in response to the delivery of a battery of gustatory stimuli. During an ingestion response, rhythmic bursts of unit activity were either in phase with the anterior digastric (protruder type activity) or out of phase with the AD and in phase with STY (retractor type activity). During rejection responses to QHCl, some neural responses showed a decrement, in contrast to the peripheral musculature.
Supported by NS 24889

473.3

SODIUM DEPRIVATION PRODUCES ALTERATIONS OF CHORDA TYMPANI TERMINAL FIELDS IN THE NUCLEUS OF THE SOLITARY TRACT IN ADULT RATS.
Camille Tessitore* and David L. Hill (SPON: P. Lasiter). University of Virginia, Charlottesville, VA.

Recordings from rat chorda tympani (CT) nerves have demonstrated that sodium deprivation on or before 8 days postconception and continued throughout development reliably produces a suppressed taste response to NaCl while taste responses to NH_4Cl and KCl are unaffected. In order to investigate whether the alteration of the peripheral response in the deprived rat is anatomically reflected in the first order synapse in the CNS, we examined terminal fields of the CT in Na deprived and Na replete animals. HRP was applied to cut nerves and the tissue was processed according to a modified TMB technique after 24 hour survival. Results indicate that while the fields of both control and deprived animals were located in the rostral pole of the NTS, control CT terminal fields were confined in a discrete ovoid configuration, while terminal fields of Na deprived animals were in a diffuse circular configuration. Preliminary results indicate that the total volume of terminal fields is increased in Na deprived animals as compared to controls. These findings indicate that the central projection areas of the gustatory system are sensitive to environmental manipulations. (Supported by NIH NS24741 & NS01215).

473.4

PARABRACHIAL NEURAL ACTIVITY DURING GUSTATORY STIMULATION IN AWAKE RATS. H. Nishijo* and R. Norgren Department of Behavioral Science, College of Medicine, The Pennsylvania State University, Hershey, PA 17033.

Rats were adapted to receiving water while in a plastic restrainer. Under anesthesia, cranioplastic acrylic was attached to their skulls and formed around 4 pins that were bolted to the stereotaxic earbars. Two intraoral catheters also were implanted. Ten days later the animals resumed a deprivation regimen in which they received their water while restrained and with their heads held securely by the acrylic cap. Single neurons were isolated in the dorsal pons with microelectrodes introduced via a previously prepared skull opening. Data were collected from 46 cells in two animals while they responded to standard sapid stimuli consisting of 0.05 ml of 0.1 M NaCl, 0.3 M sucrose, 0.003 or 0.01 M citric acid, and 0.0001 M quinine HCl, as well as to water. For a subsample of neurons, concentration response functions were generated for some (n=7) or all (n=9) of the sapid chemicals. When compared with equivolume infusions of distilled H_2O, 40 neurons responded differentially to one or more of the sapid stimuli. The remainder responded equivalently to both water and taste stimuli. Based upon their responses to the standard stimulus concentrations, the majority of neurons were most sensitive to NaCl (n=26) or to sucrose (n=9). Within these categories, 7 cells responded to a single sapid chemical (Na=4; S=3). Responses to citric acid and quinine were less frequent, sometimes confounded with apparent water responses, and except at highest concentrations, generally of lower amplitude. Supported by PHS grants NS 30397 and MH 00653.

473.5

ORAL SENSORY RESPONSES IN THE NUCLEUS OF THE SOLITARY TRACT. S.P. Travers & R. Norgen. College of Dentistry, Ohio State University, Columbus, OH 43210 & College of Medicine, Pennsylvania State University, Hershey, PA 17033.

The rostral two-thirds of the nucleus of the solitary tract (NST) receives primary afferent terminations from at least 6 separate gustatory receptor subpopulations, as well as somatosensory structures in both the rostral and caudal oral cavity. Single-unit responses were recorded from an extensive area of NST to ascertain the organization of responses arising from all oral gustatory receptor subpopulations and from mechanical stimulation of oral structures. We recorded from 17 gustatory-and 14 mechanically-responsive NST neurons; it is unclear whether any gustatory cells responded to mechanical stimulation, but none of the mechanical neurons were gustatory-sensitive. Seven gustatory cells received information from a single gustatory receptor subpopulation, such as the foliate papillae, whereas 7 others were activated by stimulating multiple receptor groups; e.g., the soft palate and foliate papillae. The remaining taste cells were sensitive only to stimulation of the entire oral cavity. Similarly, some mechanosensitive neurons were activated only by stimulating a specific region on the tongue (e.g., the circumvallate) whereas others were sensitive to both lingual and palatal stimulation. There was an orotopic map of both gustatory and somatosensory responses, with the somatosensory representation lateral and/or caudal to the gustatory.
Supported by NS24884 and NH00653.

473.6

ANTERIOR TONGUE TASTE AND TACTILE PROJECTIONS TO NUCLEUS OF THE SOLITARY TRACT (NST) IN SHEEP FETUSES AND LAMBS. C. M. Mistretta. Univ. of Michigan, Ann Arbor, MI 48109.

To determine whether there is a functional microorganization for salt taste and tactile projections from fungiform papillae on the anterior tongue to second order neurons, and whether the organization changes during development, multiunit recordings were made in the NST of sheep. Six fetuses at 130 days of gestation (term = 147 days), six perinatal animals (5 days before or after birth), and six postnatal lambs (30 to 60 days) have been studied. With an initial location in the anterior tongue taste projection, neural activity was recorded at 0.2 mm steps throughout rostral-caudal and medial-lateral coordinates. Within each electrode track, responses were recorded at 0.1 mm steps. Stimuli were 0.25 and 0.50 M NH_4Cl, NaCl, and KCl, and light touch with a glass probe. At all ages there was a trend for NaCl to elicit larger responses, relative to NH_4Cl, at more rostral NST coordinates. However, a well defined chemotopy for salts was not observed. Responses from papillae on the tongue tip contributed proportionately more to the taste response at more rostral NST locations. Throughout the nucleus, responses to salts were recorded most dorsally, then responses to salts and touch, and then, most ventrally, responses to touch only. For tactile responses, the posterior tongue was represented most dorsally and the anterior tongue most ventrally. These data indicate some somatotopy for anterior tongue taste and tactile projections in NST that is established early in development, and the absence of any well defined chemotopy for salts. (Supported by NIH Grant NS 25825.)

473.7

TASTE RESPONSES IN THE PARABRACHIAL PONS OF OVARIECTOMIZED RATS. P.M. Di Lorenzo, S. Monroe* and J. Levine*. Dept. of Psychology, SUNY at Binghamton, Binghamton, N.Y. 13901.

Since the early 1970s it has been known that female rats prefer higher concentrations of sweet stimuli compared with males. Recent data from our lab (Di Lorenzo & Monroe, in preparation) have revealed that units in the parabrachial nucleus of the pons (PbN) of diestrus female rats showed larger responses to sweet stimuli compared with PbN units in males. Because it has been shown that ovariectomized rats have lowered preferences for saccharin compared with intact females, it is possible to predict that responses to sweet stimuli in the PbN of ovariectomized rats might be of lower magnitude compared with those in intact females. To investigate this latter hypothesis, electrophysiological responses to representatives of the 4 basic taste qualities were recorded in the PbN of ovariectomized rats. Gustatory stimuli included 3 concentrations of each of the following: NaCl, HCl, saccharin, sucrose and quinine. Preliminary analysis of 32 PbN units in ovariectomized rats showed that responses to sweet stimuli were of comparable magnitude to those in female rats. However, responses of PbN units to quinine appeared to be elevated in ovariectomized rats compared with male and female rats. Multidimensional scaling analyses of PbN unit responses to the stimuli constructed a "taste space" that closely resembled that constructed by similar analyses of PbN responses in males. These results suggest that decreased saccharin prefence in ovariectomized rats may reflect a greater sensitivity to the bitter components of the taste of saccharin. Moreover, these data provide evidence that taste responses in the PbN are influenced by both the activational and organizational actions of ovarian hormones. Further analyses will focus on this issue in greater detail.

Supported by BRSG Grant S07RR07149-12 to P.M. Di Lorenzo.

473.8

MULTIMODAL CONVERGENCE WITHIN AND BEYOND THE SECONDARY TASTE CORTEX IN PRIMATES. L.L.Wiggins, E.T.Rolls and T.Chisholm*. Dept. of Exptl. Psychology, University of Oxford, England.

It has been found that there is a secondary cortical taste area in the caudolateral orbitofrontal cortex (clOFC) of the primate (Macaca fascicularis) (Rolls et al., 1985 Chem. Senses 10: 442). This area receives projections from the primary taste cortex in the frontal operculum and insula (Wiggins et al., 1987 Chem. Senses 12: 206). In this study we investigated whether the neurons in this area, and in the more medial caudal orbitofrontal cortex (OFC) which also contains taste responsive neurons (Thorpe et al., 1983 Exp. Brain Res. 49: 93), are multimodal, and receive visual and/or olfactory inputs as well as gustatory inputs. Of the neurons tested for multimodal inputs, some were found which responded to visual as well as to gustatory stimuli. The majority of these had corresponding sensitivities in the two modalities, in that they responded best to sweet tastes (e.g. 1M glucose), and responded more in a visual discrimination task to the visual stimulus which signified sweet fruit juice than to that which signified saline. In addition some neurons were found which responded to visual and to olfactory stimuli. These results show that there are regions in the orbitofrontal cortex where the sensory modalities of taste, vision, and olfaction converge; and that in the majority of cases the neurons have corresponding sensitivities across modalities.

473.9

MENTHOL ALTERS PERIPHERAL AND CENTRAL NEURAL CODING OF RAT LINGUAL TEMPERATURE SIGNALS. G.J. Schwartz* and E. Kosar (SPON.: B. Bryant) Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104.

The rat tongue is sensitive to both thermal and chemical stimulation. Peripheral nerve studies have revealed that neural activity may be elicited by chemical and thermal stimuli in peripheral tongue temperature fibers. To elucidate the neural coding of these chemical and thermal signals, we examined both peripheral nerve fiber and cortical single unit responses to warm water (WW) (40°C), cold water (CW) (18°C), 0.02% Menthol (M), and 0.001% capsaicin (C). Electrolytic lesions were used to determine the cytoarchitectural and laminar position of cortical cells responding to thermal and chemical stimulation. Peripheral nerve recordings revealed two distinct groups of fiber types; Type I, restricted to chorda tympani nerve fibers, responded with increased activity to CW stimulation, yet spontaneous activity was not altered by WW, M or C. Type II, restricted to trigeminal lingual nerve fibers, responded to CW stimulation, but this CW excitation was dramatically and significantly reduced by prior exposure to menthol. In addition, some Type II fibers were inhibited by WW and unaffected by M or C alone. Two distinct classes of cortical neurons were identified which had response patterns similar to the two types of peripheral nerve fibers found. Thus it appears that the peripheral organization of some lingual thermal input is maintained at the level of the cortex. However, other patterns of cortical responses not observed in the periphery, were also identified, revealing more complex processing of oral thermal and chemical signals.
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473.10

SINGLE UNIT ACTIVITY IN RAT GUSTATORY CORTEX. E. Kosar and G. J. Schwartz Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104

Present knowledge about neural coding of taste information in gustatory cortex is very limited. The cytoarchitectural definition of this zone has been established only recently (Kosar et al. '86). To date only a restricted number of taste stimuli have been tested for their ability to evoke cortical activity in the rat. In order to determine whether distinct populations of gustatory neurons exist at the cortical level and to more clearly establish what stimulus features they encode, it is necessary to determine the effectiveness of a wider range of chemical stimuli. In these experiments, single unit activity was recorded in the agranular insular cortex of the rat in response to stimulation of the anterior oral cavity. The stimulus set included 24 different chemicals (plus additional concentrations of some of these stimuli), as well as warm water (40°C), cold water (18°C) and tactile stimulation. The purpose of these experiments was 1) to determine the range of sensitivities of each single cortical unit recorded, and 2) to ascertain whether adjacent cortical units share similar response properties as would be predicted by a modular or columnar pattern of organization. In general, cortical units tend to be broadly tuned, responding to several categories of chemical stimulation (for example, salts and acids). Adjacent cortical neurons can differ in their responsiveness to individual tastants. Some overlap in taste sensitivities is usually observed; the most effective stimulus for eliciting a response is often the same for adjacent neurons.

Supported By NSF grant #BNS-8519629 to E.K.

473.11

GUSTATORY RESPONSES OF SINGLE NEURONS TO AN EXTENDED STIMULUS ARRAY IN GUSTATORY CORTEX OF THE ALERT MACAQUE MONKEY. T.R. Scott, and V.L. Smith*, Dept. Psychology and Inst. Neurosci., Univ. Delaware, Newark, DE 19716.

Models of gustatory neural coding have been based primarily on recordings from the hindbrains of anesthetized rodents. Where these models fail to predict human psychophysical data, three levels of ambiguity must be addressed: species differences (rodent vs human), relevant neural level (hindbrain vs cortex) and anesthetic effects. We have developed the capacity to record from single gustatory neurons in the insular-opercular cortex of the alert macaque. We now report on the responses of 41 of these cells in two monkeys to a representative array of 16 salts, acids, sugars and alkaloids, all at moderate-high concentrations. The mean spontaneous rate was 5.4±4.4 s/s (range=0.5-25.5 s/s). Of 656 trials (16 stimuli x 41 neurons), 234 (36%) elicited excitation, 19 (3%) inhibition and 403 (61%) no response. The mean breadth-of-tuning coefficient was 0.80 (range=0.38-0.99). Correlations between pairs of stimulus profiles generally confirmed expected similarities ($r_{MgCl-CaCl}$=+0.95) and dissimilarities ($r_{gluc-NaCl}$=+0.29) of taste quality. There was no compelling evidence for the segregation of taste cells into functionally discrete classes. While compromises in the precision of stimulus application and recording stability must be made these data demonstrate the feasibility of obtaining reliable records from the alert monkey.

Supported by research grant BNS 8514202 from the NSF.

473.12

ACTIVITY OF GUSTATORY CORTEX IN THE HAMSTER STUDIED WITH VOLTAGE SENSITIVE DYES. J.A. London (SPON: M. McPheeters). Dept. Bio-Structure & Function, Univ. CT Health Ctr, Farmington, CT 06032.

Optical recording methods were used to detect hamster cortical activity in response to electrical stimulation (ES) of the anterior and posterior tongue. Following a craniotomy over the gustatory cortex, a fluorescent dye, RH795, (a gift from Drs. L.B. Cohen and A. Grinvald) which changes its fluorescence with changes in membrane potential, was externally applied to the cortex. Fluorescence changes were recorded by a photodiode array. Anterior tongue ES resulted in activation of a small, (900 x 900 u) discrete cortical area. Posterior tongue ES activated a separate, more caudally located cortical area. There was also a temporal difference; cortical activity latency following anterior tongue ES was 25-60 msec, as compared to a latency of 90-150 msec following posterior tongue ES. Surrounding brain areas were not activated by anterior or posterior tongue ES. Bilateral destruction of the chorda tympani eliminated cortical activity in response to anterior tongue ES but not to posterior tongue ES. In conclusion, it appears that a discrete area of the hamster cortex which responds to tongue ES has been identified with voltage sensitive dyes. This area is bounded ventrally by the rhinal fissure and bounded dorsally by the somatosensory cortex. This area can functionally be divided into two parts: an area activated by anterior tongue ES and an area activated by posterior tongue ES. These results suggest that information to the rostral cortical area is carried by the chorda tympani. Supported by NIH grant NS16993.

474.1

NORADRENERGIC MODULATION OF OLFACTORY BULB GLYCOGEN METABOLISM. R. Coopersmith and M. Leon. Dept. Psychobiology, Univ. California, Irvine CA 92717

The rat olfactory bulb has high levels of glycogen phosphorylase, the enzyme which mobilizes glucose from glycogen. In cortex, phosphorylase is activated by norepinephrine. We therefore examined the role of norepinephrine in the control of olfactory bulb glycogen breakdown.

Olfactory bulb slices were incubated with tritiated glucose, resulting in tritiated glycogen formation. Slices were then treated with test drug, homogenized, and glycogen was extracted and measured.

Norepinephrine induced a concentration-dependent breakdown of glycogen with an ED50 of approximately 300 pM. The published value of the ED50 for norepinephrine-induced glycogenolysis in cortical slices is three orders of magnitude higher, suggesting that the olfactory bulb is an extremely sensitive target tissue for this amine.

We will also present data concerning the adrenergic receptor sub-type(s) underlying this effect. In particular, we will test the hypothesis that the sensitivity of olfactory bulb phosphorylase to norepinephrine is the result of an alpha-receptor mediated potentiation of a beta-receptor mediated response.

474.2

HISTAMINE EFFECTS ON EEG AND EVOKED POTENTIALS IN THE RAT OLFACTORY BULB. B. K. Rhoades, P. B. Cook and W. J. Freeman III. Dept of Physiology & Anatomy, Univ. of Cal. at Berkeley, Berkeley, CA 94720.

Histochemical and electrophysiological evidence has established histamine as a neuromodulator in the CNS. Histaminergic fibers originating in the tuberomammillary nuclei of the hypothalamus travel in the medial forebrain bundles and radiate widely through the forebrain. The olfactory bulbs, as cortical structures whose cytoarchitecture and electrophysiology are well characterized, are logical targets for investigation of histaminergic action.

Rats were prepared for electrophysiological recording by pentobarbital anesthesia supplemented with analgesia at surgical and stereotaxic sites. The lateral bulbar surface, primary olfactory nerve (PON) fiber bundles of the olfactory mucosa, and the lateral olfactory tract (LOT) were unilaterally surgically exposed. The PON and LOT were stimulated via bipolar electrodes. EEG and evoked potentials (EPs) were recorded from a linear array of surface electrodes oriented dorso-ventrally on the lateral bulbar surface, while the EEG of the contralateral bulb was recorded via an electrode on its dorsal surface. EPs in response to PON and LOT stimulation were recorded for near threshold stimulus intensities and averaged over 50-100 trials. Histamine at .05-5mM in an artificial cerebrospinal fluid vehicle was administered locally to the bulbar surface in the vicinity of the recording array via a push-pull cannula system.

Following histamine application EEG of the treated bulb showed a marked increase in sinusoidal burst activity. Averaged PON EPs showed an attenuation of the damped sinusoidal component and a marked attenuation of the negative slow wave component. Averaged LOT EPs showed a marked decrease in damping and a slight decrease in oscillation frequency. These effects are similar to those previously observed on administration of mecholyl with prostigmine and may be attributed to a steady state increase in periglomerular cell activity. This would attenuate PON glomerular throughput, as well as tonically increasing the excitability of the mitral-tufted cells. Current research is focused on identification and localization of the relevant histamine receptor type(s) as well as elucidation of the ionic mechanisms involved.

Supported by MH06686

474.3

EVIDENCE FOR GABA-MEDIATED INHIBITION IN THE SALAMANDER OLFACTORY BULB. K.A. HAMILTON, S.R. NEFF and J.S. KAUER. Neuroscience Program and Dept. of Neurosurgery, Tufts-New England Medical Center, Boston, MA 02111.

In the olfactory bulb, GABAergic interneurons, many of which are granule cells, exert powerful control over the excitability of mitral/tufted output cells. In response to electrical and odor stimulation, this control is manifest as feedback inhibition mediated through reciprocal dendro-dendritic synapses. As a first step toward understanding how odor responses are generated in the olfactory bulb, we have used a variety of techniques to examine how GABAergic, granule-layer cells might control mitral/tufted cell excitability in the salamander.

Intracellular recordings obtained from mitral/tufted cells in the salamander have shown that a prolonged biphasic IPSP, similar to that in the rabbit and turtle, follows electrical stimulation (Hamilton and Kauer, J. Neurophys. 59: 1988). Immunocytochemical staining has also provided evidence that granule-layer cells, as well as other interneurons, are GABAergic. The effects of these cells on mitral/tufted cell activity has also been assessed using GABA blockers during video-rate imaging of voltage-sensitive dye fluorescence (Kauer, Nature 331:166, 1988). We are now applying the combination of these methods to the analysis of how other components of the olfactory bulb circuits might contribute to the generation of responses elicited by odor and electrical stimulation. Supported by grants NS-22035, NS-20003 and the Dept. of Neurosurgery.

474.4

EARLY AND LATE RESPONSES IN SLICES OF SALAMANDER OLFACTORY BULB: OPTICAL RECORDING OF ELECTRICAL EVENTS THAT DEPEND UPON CA^{++}. A.R. Cinelli* and B.M. Salzberg. Univ. of Penn., Phila., PA 19104.

Fast and slow extrinsic optical signals have been reported in several olfactory bulb preparations. The former reflects the compound action potential, but the origin of the slow signal is less certain. Optical recordings (RH-155; 0.3 - 0.5 mg/ml) from tiger salamander slices exhibited similar depolarizing responses to orthodromic stimulation. Moderate stimuli often generated an additional long-latency optical signal.

The slow signal that followed stimulation of the glomerular layer was reduced in size by Cd^{++} (50-100 μM), and in the presence of low Ca^{++}, while replacement of Ca^{++} by Sr^{++} increased its size and duration. Neither the fast nor the slow optical signals observed at the level of the mitral cell and granular dendrites, were abolished by TTX (5 μM) or 0 $[Na^{+}]_o$. These observations, together with the results of experiments using GABA, Baclofen, and APV, suggest the presence of a longlasting excitation at mitral cell dendrites which depends upon Ca^{++}, and which gives rise to the slow optical signals.

Supported by USPHS grant NS 16824 and a Fogarty Fellowship to ARC.

474.5

HIGH RESOLUTION VIDEO IMAGING OF ODOR RESPONSES IN THE SALAMANDER OLFACTORY SYSTEM. J.S.KAUER and S.R. NEFF Neuroscience Program and Dept. of Neurosurgery. Tufts-New England Medical Center, Boston, MA. 02111.

Global events simultaneously occuring in neuronal elements that have been activated in parallel can be measured by video-rate imaging of voltage-sensitive dye fluorescence (Kauer, Nature, 1988). Using this method, patterns of electrically-elicited activation in the tiger salamander olfactory bulb have been observed. In the present study, we show an improvement in temporal resolution from 33 to better than 16 ms/video-frame and report an analysis of responses obtained after odor stimulation of the olfactory receptors. Stimulation with one-half second pulses of odor elicits widespread, temporal patterns of depolarization and hyperpolarization in different bulbar layers which are similar to those seen in the same layers using intracellular recording. The distribution of activity within the layers is distinctively different for different odors. These data provide additional evidence that odor information is encoded by patterns of activity distributed in time and space across the cells at each level of the olfactory pathway and have begun to allow a quantitative assessment of the stability of the patterns across animals. Initial experiments are in progress in which we have imaged activity in the olfactory receptor cell population after both odor and electrical stimulation.

Supported by grant NS-20003 and the Dept. of Neurosurgery.

474.6

NEUROBEHAVIORAL CORRELATES OF POSTNATAL OLFACTORY CONDITIONING: TEMPORAL CONSTRAINTS ON THE CS - UCS RELATIONSHIP. R.M. Sullivan, D.A. Wilson and M. Leon, Dept. Psychobiology, University of California at Irvine, 92717.

Acquisition of conditioned responding and learned odor preferences during olfactory classical conditioning in rat pups requires forward pairings of the conditioned stimulus (CS) and the unconditioned stimulus (UCS), i.e., the CS must precede and overlap temporally with the UCS [Sullivan & Hall, 1988]. Other temporal relationships between the CS and UCS do not result in learning. The present study was an examination of the influence of CS - UCS temporal relationship on the acquisition of modified olfactory bulb neural responses to the CS.

Wistar rat pups were trained (PN1 - PN18, 10 min/day) with either forward (odor and stroking) or backward (stroking then odor) CS-UCS pairings. On PN19, pups were tested for a behavioral odor preference to the CS, or for olfactory bulb ^{14}C 2-DG uptake or single-unit responses to the CS.

Only pups with forward CS-UCS pairings demonstrated modified behavioral and neural responses to the CS. Thus, these results suggest that, as for the behavioral responses, modified olfactory bulb neural responses are changes specific to associative learning. Formation of this neural association requires temporal constraints on the CS - UCS relationship. [Supported by NS26100 to RMS, BNS8606786 to DAW and ML, and MH00371 to ML]

474.7

MODIFICATION OF OLFACTORY BULB PHYSIOLOGY FOLLOWING EARLY OLFACTORY DEPRIVATION. K.M. Guthrie, D.A. Wilson and M. Leon, Dept. Psychobiology, Univ. California at Irvine 92717.

Olfactory deprivation during postnatal development results in profound structural and neurochemical modification of the olfactory bulb. The present report is a description of the neurophysiological effects of this olfactory deprivation.

Wistar rat pups had a single naris occluded at 2 days after birth. At postnatal day 20-22, animals were anesthetized, the occluded naris was reopened, and the previously opened naris was closed. The functioning of the primary output neurons of the deprived bulb, mitral/tufted cells, and of the major inhibitory interneurons, granule cells, was assayed using paired-pulse stimulation of the lateral olfactory tract. Paired-pulse stimulation provides a measure of granule cell-mediated inhibition of mitral cells. In addition, olfactory bulb responses to odor were assessed using both single-unit recording from mitral/tufted cells and ^{14}C 2-deoxyglucose autoradiography. Following neurophysiological recordings, olfactory bulbs were processed for tyrosine hydroxylase immunoreactivity, to ensure that the bulb had been deprived. Olfactory deprivation has been previously shown to reduce TH-IR in juxtaglomerular neurons.

The results suggest that although levels of granule cell-mediated inhibition appear immature after early deprivation, the deprived bulb is functionally capable of responding to odors.

[Supported by grants MH09635-01 to KMG, and BNS8606786 to DAW and ML.]

474.8

SPATIAL PATTERNS OF MITRAL/TUFTED CELL RESPONSES TO ODORS INVESTIGATED WITH COMBINED SINGLE-UNIT, HRP AND ^{14}C-2-DG TECHNIQUES IN THE RAT. D.A. Wilson and M. Leon, Dept. Psychobiology, Univ. California at Irvine 92717.

Odor-specific spatial patterns of radiolabeled 2-DG uptake can be observed in the olfactory bulb glomerular layer. The present study attempted to correlate these spatial patterns of metabolic activity with activity of olfactory bulb output neurons, mitral/tufted (M/T) cells.

Extracellular single-unit recordings were made from M/T cells in regions typically associated with 2-DG uptake to peppermint odor. Responses to peppermint and isoamyl acetate at 2 different intensities were monitored. After the recording session, HRP (4% in 0.5M KCl) was iontophoresed from the recording pipette. The animal was allowed to recover 24 hr, and was then injected with ^{14}C-2-DG and exposed to peppermint odor for 45 min. Alternate sections of the olfactory bulb were processed for 2-DG autoradiography (20 um sections) or HRP histochemistry (60 um sections; Hanker-Yates or TMB histochemistry). Apical dendrites of M/T cells near the recording site were reconstructed into the glomerular layer and compared with regions of focal glomerular 2-DG uptake.

Preliminary evidence suggests that response characteristics of M/T cells vary with spatial location in the bulb. This spatial pattern of M/T cell responses appears to correspond with spatial patterns of glomerular layer 2-DG uptake. [Supported by BNS-8606786 to DAW and ML.]

474.9

CSD ANALYSIS OF OSCILLATORY RESPONSES IN RAT PIRIFORM CORTEX REVEALS STEREOTYPED CYCLICAL COMPONENTS MEDIATED BY AFFERENT AND INTRINSIC ASSOCIATION FIBERS. K.L.Ketchum* and L.B.Haberly. Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

CSD analysis was performed on the posterior piriform (olfactory) cortex of urethane anaesthetized rats. The response to high strength lateral olfactory tract (LOT) shocks was similar to that observed in the opossum (Haberly, Chem. Senses 10:219). CSD analysis during period 1 (initial surface negative component) of the field potential evoked by high strength shocks revealed a sequence of inward membrane currents correlated in depth with afferent and intrinsic fiber systems. The first inward current (sink) occurred at the depth of afferent fiber terminals on the distal apical dendrites of superficial pyramidal cells (SP cells). Then, two successively deeper sinks appeared in layer Ib at the depths of the intrinsic association fiber terminals on the mid and proximal segments of SP cell apical dendrites. During this time, a small sink usually occurred in layer III at the depth of SP cell basal dendrites and deep cells.

Low strength shocks evoked damped oscillatory field potentials with a period of approximately 20ms, lasting 200-300ms. These oscillations superficially resemble those recorded in response to odor stimulation. CSD analysis of these responses to low strength shocks revealed a stereotyped sequence of events that recurred during each cycle of the oscillation. Within each cycle a sink occurred in layer Ia, followed by two successively deeper sinks in layer Ib. These sinks occurred at the same depths as in the high strength shock responses and with similar latency, but were of shorter duration. This result raises the possibility that a stereotyped sequence of synaptic events, evoked by afferent and intrinsic association fiber systems, occurs repetitively at approximately 50Hz during the oscillatory response to natural odor stimulation. Supported by NINCDS grants NS08328 to KLK and NS19865 to LBH.

474.10

INTEGRATION OF COMPUTER SIMULATIONS AND MULTIUNIT RECORDING IN THE RAT OLFACTORY SYSTEM U.S.Bhalla*, M.A.Wilson*, J.M.Bower. Division of Biology, Caltech, Pasadena CA 91125 (SPON. R. Sinsheimer)

For the last several years we have been developing a component-level model of piriform cortex based on electrophysiological and neuroanatomical data. The model replicates neural responses obtained from experimental manipulations (e.g. shock stimulation of the LOT). We are now exploring the capabilities of this network for learning and classification of olfactory stimuli. These studies require information concerning the representation of information at the level of the olfatory bulb. We are using recordings of multiple single neuron activities during behavioral tasks to provide data about network-level processing within the bulb, and about the form of the output to higher processing areas like the piriform cortex. To obtain this type of data we have implanted multiple glass-coated platinum-iridium electrodes in the olfactory bulb. We are also developing methods for implantation of multi-channel silicon electrodes developed in our lab (Nelson, Rasnow, Banik and Bower, Soc. Neurosci. Abs. this volume). This multi-cell data, in conjuction with structural simulations of the bulb and piriform cortex, is being used to make predictions about the network properties of these areas and to elucidate the processing tasks carried out by them. (work supported by NIH grant NS22205, NSF grant EET-8700064, the Whitiker Foundation, and the Joseph Drown Foundation.)

474.11

DEPENDENCE OF PARTITIONING BEHAVIOR OF AN ANATOMICAL MODEL OF PIRIFORM CORTEX ON PHYSIOLOGICAL VARIABLES. M.W.Jung*, G.Lynch, R.Granger* (SPON: Y.Torigoe). Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA. 92717.

Coherent aggregate behavior of a biological simulation of piriform (olfactory) cortex, based strictly on its macro- and microanatomical architecture, cell biophysics, physiology, and synaptic transmission and synaptic modification rules corresponds to unsupervised partitioning of simulated input cues into similarity-based clusters (Lynch and Granger, *Psych.Learn. & Motiv.*, 1988). Experimental manipulation of physiological features comprising the simulation identifies their effects on its partitioning behavior; the resulting predictions of values of these biological parameters are directly testable physiologically. Initial investigations (see, e.g., Granger et al., IEEE NIPS Proceedings, 1987) showed that formation of robust clusters depends on incremental potentiation step size in the range of 5% of distance between naive and potentated synaptic weights. In an experiment investigating the effects of varying this distance, the distance was set at values ranging from 25%–100% increase. The major effect was in the breadth of partitions that could be formed by the network: smaller weight distance led to broader clusters. Average angle ϕ between input cue vectors within a learned cluster measures the breadth of the cluster: smaller ϕ indicates that more similarity among cues was necessary for clustering. Average angle ϕ is a monotonically increasing function (within the range $0° - 90°$) of the ratio of potentiated to naive synaptic weights ω_2/ω_1. A related experiment investigated the effect of altering the relative efficacy of naive and potentiated synapses during learning episodes. Potentiated synapses do not contribute significantly more conductance then naive synapses when activated in the theta-burst mode necessary for inducing synaptic change; with this feature in the network, multiple categories are learned with little interference; otherwise, interference appears among learned categories due to 'attractor' effects of potentiated cells during learning of inputs in different categories from those cells. These and related experiments with this simulation have led to a deeper understanding of the direct relationships between biological variables and aggregate network behavior, and have permitted simplification of the network to tractable form, leading to theoretical analysis that has identified deep relations among physiological variables (Granger, Ambros-Ingerson and Lynch, 1988, in press).

Supported by ONR # N00014-87-K-0838 and NSF # IST-85-12419.

474.12

CIRCUITRY AND SYNAPTIC MECHANISMS UNDERLYING SELECTIVE LONG-TERM POTENTIATION IN PYRIFORM CORTEX: A HYPOTHESIS. D. K. Patneau and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Previous research in our laboratory has demonstrated a long-term potentiation (LTP) of the pyriform cortex (PC) potential evoked by olfactory bulb (OB) stimulation which is selective to late components of the evoked potential and appears to be functionally inhibitory (Soc. Neurosci. Abstr. 11: 780, 1985). This selective potentiation can be produced by repeated high-frequency stimulation of the OB or the association fiber system in the PC, but not by direct activation of the lateral olfactory tract, the major afferent pathway from the OB to the PC. This, and other evidence to be presented, strongly implicates the association fiber system in the PC as the pathway mediating selective LTP. Further evidence indicates that production of selective LTP requires the activation of NMDA receptors.

A model of the circuitry and synaptic mechanisms underlying selective LTP will be presented. Discussion will focus on the ability of the model to account for the characteristics of selective LTP as documented by research in our laboratory and the potential involvement of this form of plasticity in information processing in the olfactory forebrain.

Supported by NSF Grant BNS 85-19700 and the Marie Wilson Howells Fund.

474.13

ROLE OF ASSOCIATION FIBERS IN SELECTIVE LONG-TERM POTENTIATION IN THE PYRIFORM CORTEX. J. S. Stripling and D. K. Patneau. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Electrical stimulation of the olfactory bulb (OB) or lateral olfactory tract (LOT) elicits an evoked potential in the pyriform cortex (PC) whose initial wave (period 1) reflects activation of PC pyramidal cells via the LOT and subsequent excitation within the PC. This is followed by period 2, which is associated with inhibition of PC pyramidal cells. Repeated high-frequency stimulation of the OB produces a selective long-term potentiation (LTP) of period 2 (Brain Research 441: 281-291, 1988), while stimulation restricted to the LOT does not (Soc. Neurosci. Abstr. 12: 508, 1986).

In the present experiment male Long-Evans rats with chronically implanted electrodes received repeated high-frequency stimulation of either the LOT, layer Ib, or layer III of the PC. Stimulation of either layer Ib or layer III produced a selective LTP of period 2 in the PC similar to that produced by OB stimulation in previous studies. Stimulation of the LOT produced minimal effects. These results suggest that activation of the association fiber system which runs in layers Ib and III of the PC is critical for the production of LTP. An accompanying presentation (Patneau and Stripling) will present a model of LTP in the PC which incorporates these findings. (Supported by NSF Grant BNS 85-19700.)

476

Symposium. FORM AND SYNAPTIC FUNCTION IN RETINAL GANGLION CELLS. E.V. Famiglietti, University of Calgary (Chairperson); R.W. Rodieck, University of Washington (Co-chairperson); J.I. Simpson, New York University; H. Wässle*, Max Planck Institute (Frankfurt); N.W. Daw, Washington University.

Recent advances in anatomical, physiological and pharmacological techniques have converged in the study of retinal ganglion cells (GCs). As a result, strong hypotheses have emerged concerning the neural circuitry underlying well defined physiological responses. Some investigations have included "identified" GCs with input to well-studied visual centers of the brain. The intent of this symposium is to focus on recent work in mammals, bringing together evidence on functional circuits obtained via several different approaches. Bob Rodieck will describe the relationship between dendritic form and central projections in intracellularly stained cat and monkey GCs. Jerry Simpson will explain the functional role of directionally selective (DS) information in the accessory optic system, innervated by morphologically and physiologically identified rabbit GCs. Ted Famiglietti will describe ultrastructural studies on the organization of synaptic inputs to identified DS GCs in rabbit. Heinz Wässle will present structural and pharmacological evidence on control of the rod pathway to GCs in cat, and Nigel Daw will outline the effects of 4 major groups of neurotransmitters upon physiological properties of rabbit GCs, and the neural circuits involved.

477

SYMPOSIUM. NEUROPEPTIDES, STEROIDS AND BEHAVIOR. G.F. Koob, Scripps Clinic (Chairperson); L.W. Swanson, Salk Inst.; P.M. Plotsky, Salk Inst.; D.W. Pfaff, Rockefeller Univ.; A.N. Epstein, Univ. of Pennsylvania.

This symposium will discuss neuropeptides, steroids and their interactions in functions of the CNS. We will address roles of brain neuropeptides in mediating steroid effects, and mechanisms for steroids to alter brain functions, including behavior. Specific presentations will include CRF, LHRH and preproenkephalin as they control pituitary function and extra-hypothalamic circuits.

ENDOCRINE CONTROL AND DEVELOPMENT IV

478.1

ORGANIZATION OF ARGININE VASOPRESSIN AND OXYTOCIN CELLS AND FIBERS IN THE HYPOTHALAMUS AND ANTERIOR PITUITARY OF THE FETAL SHEEP LATE IN GESTATION. G.E. Hoffman, T. McDonald, J.P. Figueroa and P.W. Nathanielsz, Department of Physiology, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261 and Lab. for Preg. and Newborn Res., Cornell Univ., NYS Coll. of Vet. Med., Ithaca, NY 14853.

Arginine vasopressin (AVP) and oxytocin (OXY) participate along with corticotropin releasing factor (CRF) in the hypothalamic-pituitary-adrenal axis. In the fetal sheep, adrenal stimulation can induce premature delivery and adrenal activity increases after 130 days gestation (d GA). This suggests that changes in the adrenal axis during the latter part of gestation dictate the onset of parturition. The aim of the present study was to examine the organization of vasopressin and oxytocin-immunoreactive structures in the fetal sheep hypothalamus at this critical period.

Six Rambouillet-Columbia sheep fetuses, delivered by cesarian section under Bio-Tal anesthesia at 134 to 145 d GA., were perfused with Zamboni's fixative and their brains processed for localization of AVP, OXY, or neurophysin (NP) using the ABC method. AVP and OXY within the magnocellular hypothalamic nuclei and the hypothalamo-neurohypophyseal tract were well developed. There were fewer OXY cells than AVP neurons and they were often segregated. Projections from the PVN joined the fibers from the SON and accessory nuclei in the hypothalamo-neurohypophyseal tract to the median eminence and posterior pituitary. AVP and OXY fibers of the internal zone of the median eminence continued into the neural lobe of the posterior pituitary. The median eminence contact zone contained a dense plexus of AVP axons and scattered OXY axons. Some AVP and OXY fibers extended into the pars tuberalis. A few AVP fibers could be further traced into the pars distalis, where they appeared to terminate in association with ACTH cells. Within the pituitary stalk, scattered AVP and OXY axons extended into the intermediate lobe. The roles of adenohypophyseal AVP and OXY are uncertain, but these axons within the adenohypophysis may provide an alternative or synergistic mechanism in the control of ACTH secretion apart from the neural-hemal route. Supported by NIH Grants RO-1 HD 18418, PO-1 HD 21350 and RO-1 AM 16166.

478.2

ORGANIZATION OF CORTICOTROPIN RELEASING FACTOR CELLS AND FIBERS IN THE HYPOTHALAMUS OF THE FETAL SHEEP LATE IN GESTATION. P.W. Nathanielsz*, T. McDonald*, J.P. Figueroa* and G.E. Hoffman (SPON: A. Humphrey). Laboratory for Pregancy and Newborn Res., Cornell Univ., N.Y. State Coll. of Vet. Med., Ithaca, NY 14853 and Department of Physiology, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

Corticotropin releasing factor (CRF) is present in fetal sheep neurons as early as 90 days gestation (d GA) and projections to the external zone of the median eminence are dense by 132 d GA (Levidiotis et al, Neuroendocrinology 46: 453, 1987). Activity of the adrenal axis shows dramatic changes after 130 d GA, suggesting that further maturation of the CRF system takes place. The aim of the present study was to examine the organization of corticotropin releasing factor (CRF) immunoreactive structures in the fetal sheep hypothalamus at this critical period of development.

Six Rambouillet-Columbia sheep fetuses, delivered by cesarian section at 134 to 145 d GA., were perfused with Zamboni's fixative under Bio-Tal anesthesia and their brains processed for localization of CRF using the ABC method. CRF was present within the paraventricular nuclei and the median eminence at all fetal ages. The paraventricular nuclei of the fetal sheep, which are C-shaped structures with their openings directed rostrally, did not display a homogeneous distribution of CRF neurons. In the younger fetuses, CRF cells were located primarily in the dorsal portion of the nucleus. In the later fetal ages CRF neurons were found in both the dorsal and ventral components of the parvocellular portions of the PVN The median eminence contact zone contained a dense plexus of CRF axons even in the youngest fetuses. Yet, the density of terminals of the CRF axons in this area increased in the older fetuses. This suggests that changes in the expression of CRF may reflect critical maturation of this neuroendocrine system prior to parturition Supported by NIH Grants RO-1 HD 18418, PO-1 HD 21350 and RO-1 AM 16166.

478.3

PROLACTIN DOES NOT EFFECT POSTNATAL BRAIN DEHYDRATION IN THE PRETERM RABBIT. A.V. Lorenzo, R.M. Beatty*. K.R. Winston* and V.C. Boss Dept of Neurosurgery, Children's, and Brigham and Woman's Hospitals, Boston, MA 02115.

Postnatal dehydration in preterm infants may lead to intracranial hypotension and to germinal matrix (GM) and intraventricular hemorrhage (IVH). Coulter et al. (Pediatr Res 19:1322, 1985) reported that prolactin (prl) reduces postnatal tissue dehydration and the incidence of GM and IVH following hypovolemic hypertension in term newborn beagles. We studied the effect of prl on tissue hydration of preterm rabbits 20% of which exhibit spontaneous GM and IVH. Bromocriptine (bro, 1 mg/kg), fluphenazine (flu, 1 mg/kg), ovine-prl (o-prl, 30.5 IU/kg) or buffer (buf) were administered daily to newborn preterm rabbits for 3 days at which time they were killed. Tissue water and serum prl levels were compared to values of littermates killed on day of birth (dob). Between dob and 3 days, the decline in respective water contents of brain, lung, skin and muscle was similar for all animals, even in those treated with o-prl in which serum prl was 8 times higher than bro, flu or buf treated rabbits. Paradoxically, serum prl levels were similar in pups treated with agents that stimulate (flu), inhibit (bro) or do not effect (buf) pituitary release of prl. We conclude that unlike the effect on term animals changes in serum prl do not alter postnatal tissue dehydration in the preterm animal. (Supported in part by NICHHD Grant 15304 and the Vallely Family Fund.)

478.4

SEXUAL DIMORPHISM OF HYPOTHALAMIC PEPTIDE SIGNALING IN REGULATION OF PULSATILE GROWTH HORMONE SECRETION. J.C. Painson* and G.S. Tannenbaum. McGill University-Montreal Children's Hospital Res. Inst., Montreal, Quebec H3H 1P3.

A striking sexual dimorphism exists in the pattern of growth hormone (GH) secretion, however, the role of the CNS in mediating this sex difference is unknown. In the present study, we examined the involvement of the two hypothalamic GH-regulatory peptides, somatostatin (SRIF) and GH-releasing factor (GRF). In freely-moving male rats, the GH response to 1 μg rGRF(1-29)NH_2 iv was significantly greater at peak compared to trough times, the latter due to antagonization by the cyclic increased release of SRIF. In contrast, females failed to exhibit a time-dependent difference in GH responsiveness to GRF suggesting that the pattern of hypothalamic SRIF secretion in females does not follow the male ultradian rhythm. Passive immunization with a specific antibody to rGRF obliterated spontaneous GH pulses in both males and females; moreover, in females anti-rGRF attenuated GH trough levels indicating a physiologic role for GRF in maintaining the elevated GH baseline of females. Hypothalamic immunoreactive GRF content was significantly lower in females compared to males (587.0±26.1 vs. 792.0±79.5 pg/fragment; $P<0.02$). Conclusions: the sexual dimorphism of GH secretion is likely due to different temporal patterning of hypothalamic GRF/SRIF signals to somatotrophes with females exhibiting tonic, rather than episodic, SRIF secretion compared to males.

478.5

A ROLE FOR THE ADRENAL IN THE MATURATIONAL PROCESS OF THE LHRH (LH-RELEASING HORMONE) NEURON. A.Barnea, G.Cho* and D.E.Hartter, Depts. OB/GYN and Physiol., Univ. Tx. Southwestern Med. Center, Dallas, TX. 75235, USA.

It is known that androgens regulate the function of the hypothalamic LHRH neuron of the male rat. It appears that the adrenal is the primary source of circulating testosterone in the immature rat and the testis is the primary source in the adult. We have previously established that the sequential exposure of median eminence (ME) explants to copper and prostaglandin E_2 (Cu/PGE) leads to a robust release of LHRH. To ascertain the role of the adrenal and testis in the regulation of the LHRH neuron during maturation, LHRH release in response to Cu/PGE (pg/15 /ME) and LHRH content (ng/ME) were assessed in ME explants of 6 wks (maturing) and 12 wks (adult) male rats 12 days after adrenalectomy (ADX) or castration (TX).

Maturing	Sham	ADX	TX	Adult	Sham	ADX	TX
Release	26.8	14.7	9.5	Release	31.1	31.1	7.9
Content	2.5	2.4	1.1	Content	3.0	2.9	1.3

TX leads to a comparable reduction in LHRH release and content; this occurs in both age groups. ADX leads to a marked reduction in LHRH release but not in LHRH content; and this occurs only in the maturing rat. Thus, the adrenal and testis exert a differential effect on the LHRH neuron. The testis regulates both the secretory and storage functions of the LHRH neuron independently of maturational age. The adrenal regulates the secretory function of the LHRH neuron in an age-dependent manner, suggestive of a change in the profile of steroids secreted by the adrenal and/or a change in the responsiveness of the LHRH neuron to a given steroid.

478.6

SEXUAL DIFFERENTIATION OF THE RAT ARCUATE NUCLEUS NEURONAL PLASMA MEMBRANE. L.M. Garcia-Segura[1]*, J. Perez[1]*, P.A. Tranque[1]*, G. Olmos[1]* and F. Naftolin[2]*, (SPON: P. Rakic) (1) Instituto Cajal, C.S.I.C., 28006 Madrid (Spain) and (2) Dept. of Obstetrics/Gynecology, Yale Univ. School of Med., New Haven, CT.

Hypothalamic sexual differentiation is controlled by estrogen. The basis for this appears to be the secretion of testosterone by the testis and its aromatization in the hypothalamus. There is a sex difference in the numerical density of intramembranous protein particles (IMP), which in adults is abolished in concert with increased endocytotic activity during estrogen treatment (J. Ster. Bioch., 29:215, 1988). EXPERIMENT: The number of exo-endocytotic images and the number of IMP in the neuronal membrane were quantitatively assessed in freeze-fracture replicas of arcuate nucleus from birth to adulthood. As before, arcuate neurons from females contained significantly more particles in their plasma membranes than neurons from males. Testosterone treatment of females resulted in an increased number of exo-endocytotic images and in the abolishment of the sex difference in the number of IMP. CONCLUSIONS: Neonatal testosterone treatment induces changes in perikaryal membrane composition. As in adults these changes may reflect an increased endocytotic activity induced by locally formed estrogen that may underlie sex differences in neuronal membrane IMPs. (Supported by CSIC, Spain and NIH-HD13587).

478.7

PLASMA TESTOSTERONE DELAYS ONSET OF MATURE BINOCULAR VISION IN HUMAN INFANTS. R. Held, J. Bauer, and J. Gwiazda, MIT Infant Vision Laboratory, Cambridge, MA 02139.

The ages of onset (average 3.5 months) of stereopsis and the fusion-rivalry discrimination measured behaviorally are delayed by several weeks in males compared to females. In contrast, no sex differences are found in the developmental course of grating acuity. This suggests that it is visual mechanisms at the cortical level which suffer delayed maturation in males. Indeed, it is during this period of development when radical changes in neuronal connectivity are occurring in the visual cortex: synaptogenesis is at its peak rate and the segregation of the ocular dominance columns is in progress. Just prior to this period, the average levels of testosterone (T) rise to a peak (about 8 weeks of age) in males and then subside. T-levels in females of this age are almost zero. To assess the role of T in modulating these neuronal developments, serial measures of T-levels were made in 12 male infants from 6-8 weeks of age until the onset of fusion-rivalry discrimination (13.6 weeks, range 9-18 weeks). This discrimination is a precise marker of the onset of mature binocularity. The correlation of the onset age of fusion-rivalry discrimination with T-levels at 8 weeks of age yields a Pearson $R=0.719$ ($p<.01$). Measurements of other steroids showed no significant correlation.

478.8

IN VIVO UPTAKE OF ^{3}H-ESTRADIOL BY THE FETAL PRIMATE BRAIN. **R.P. Michael, R.W. Bonsall* and H.D. Rees. Dept. Psychiatry, Emory Univ. School of Medicine, and Georgia Mental Health Inst., 1256 Briarcliff Road NE, Atlanta, GA 30306.**

Neurons in the preoptic area, hypothalamus, and amygdala of fetal macaques have been shown by us to concentrate radioactivity after the in vivo administration of ^{3}H-testosterone, and a major portion of the nuclear radioactivity in these regions is in the form of ^{3}H-estradiol. To study the uptake and binding of estradiol by the fetal brain, 500 uCi ^{3}H-estradiol was administered via the umbilical vein to rhesus and cynomolgus monkeys (2 males and 3 females) on day 122-125 of gestation. The fetus was delivered 1 h later by cesarean section. The brain was frozen for thaw-mount autoradiography and samples of cerebral cortex were used for analysis of radioactivity in nuclear and supernatant fractions by high performance liquid chromatography. There were only a few weakly labeled neurons in the hypothalamus and amygdala of one female, and no labeled cells in the brains of the remaining four fetuses, despite high levels of radioactivity in plasma. ^{3}H-estradiol was more rapidly cleared from fetal blood than from adult blood but was still detectable in plasma 60 min after injection. In supernatant fractions, no ^{3}H-estradiol could be detected, suggesting that the absence of labeled neurons in autoradiograms might have been due both to rapid clearance and an inability to pass from plasma to cytoplasm and, hence, into the nucleus. The mechanisms preventing the entry of estradiol into neuronal nuclei may protect the fetal brain from any deleterious effects of estrogenic hormones during development. (Supported by USPHS Grant MH 40420 and by the Georgia Department of Human Resources.)

478.9

DEVELOPMENT OF SEXUALLY DIMORPHIC AXON NUMBERS IN THE LARYNGEAL NERVE OF XENOPUS LAEVIS. Darcy B. Kelley and Jane Dennison*, Department of Biological Sciences, Columbia University, New York, N.Y. 10027

In clawed frogs, male-specific courtship song reflects activity of the more numerous laryngeal motor neurons. We examined the ontogeny of this sex difference by counting motor axons in EM photomicrographs of laryngeal nerves.

Adult males have approximately 100 more laryngeal axons than females (male: 321, female: 225). At tadpole stage 56, axon number is high (614) in both sexes. Axon number then decreases in females until the adult value is reached at 6 months post-metamorphosis. In males, 100 additional axons are added between tadpole stages 59 and 62. Whereas the extent of myelination is the same for both sexes at all stages, the number of degenerating axon profiles is higher in females. The percentage of growth cone-like axonal profiles in males declines between stages 56 to 59 and again between stages 62 to 66.

We conclude that the adult male/female difference in laryngeal nerve axon number is due to the addition of approximately 100 axons between stages 59 and 62 in males and the maintenance of this relative difference thereafter. Morphological evidence indicates (1) less cell death in males than in females and (2) outgrowth of more axons in males. Axon number is dimorphic prior to sexual differentiation of muscle fiber number and may contribute to the additional myogenesis in male larynx (Sassoon and Kelley, Am. J. Anat., 1986). Supported by NS 23684.

478.10

ISOLATING DEVELOPMENTALLY IMPORTANT GENES FROM THE LOBSTER NERVOUS SYSTEM. L.N. Geller*, L. Kobierski*, H. Potter, and E.A. Kravitz. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

In the lobster, Homarus americanus, dramatic changes in form and behavior occur during development that are paralleled by changes in the nervous system. It is likely that amines and certain peptides play important roles in some of these developmental transitions. In addition, the molting hormone, 20-hydroxyecdysone is released into the circulation of insects and crustaceans before each molt, and contributes to the transition to the next developmental stage. To facilitate the examination of nervous system development, we are attempting to clone two families of lobster genes: one represents direct transcriptional regulators of development; the other consists of proteins likely to be targets of transcriptional regulation. In the first category, we are attempting to clone the gene for the lobster 20-hydroxyecdysone receptor. We are using the zinc finger region of a hormone receptor from Drosophila melanogaster (kindly supplied by W. Seagraves) to probe a genomic library constructed from a partial Mbo 1 digest of lobster DNA cloned into EMBL-4. In the second category, we are using a probe from the active site region of the rabbit tryptophan hydroxylase gene (kindly supplied by S.L.C. Woo). Positive lobster DNA clones have been isolated using both probes and are in the process of being analyzed for comparison with other species and for their pattern of expression during lobster development.
(Supported by the NIH).

478.11

TESTOSTERONE STIMULATES NEUROPEPTIDE Y LEVELS IN SELECTED HYPOTHALAMIC SITES: EFFECTS OF AGING. A. Sahu*, S.P. Kalra, W.R. Crowley and P.S. Kalra (SPON:K.M. HEILMAN). Ob/Gyn UF Col Med, Gainesville, FL and U TN Col Med, Memphis,TN

Neuropeptide Y (NPY) is localized in various hypothalamic (HYP) sites that are innervated by LHRH-containing and gonadal steroid-concentrating neurons. Previous evidence that NPY stimulates LHRH release in steroid-primed rats and castration (CAST) decreases the release and levels of NPY selectively in 3 HYP sites, suggested that, similar to the effects on LHRH, steroids may also influence NPY neurosecretion. To test this hypothesis we examined the effects of testosterone (T) on NPY levels in microdissected HYP sites of young (2-3 mo) and old (15 mo) male rats. Young: Of the 7 sites examined, NPY levels decreased after CAST only in the median eminence (ME), arcuate (ARC) and ventromedial nucleus (VMN). Physiological T replacement immediately after CAST prevented the decrease in these sites. Further, T replacement for 10 days, beginning 2 weeks postCAST when NPY stores were depleted, significantly raised NPY levels selectively in these 3 sites. Aged: Overall NPY concentrations in each of the 7 nuclei examined were lower than those observed in young rats. As in young rats, CAST decreased NPY levels further in ARC and VMN of old rats. However, CAST also led to a decrease in NPY levels in the medial preoptic area (MPOA) and dorsomedial nucleus, but not in the ME of old rats. Also, T replacement was not as effective because the CAST-induced decrease was prevented only in the MPOA of aged rats. Thus, despite the widespread distribution of NPY in the hypothalamus, the facilitatory effects of T on NPY levels are restricted to only those sites that are involved in LHRH secretion and the aging process per se attenuated these T effects. (HD 11362).

478.12

DIFFERENTIAL EFFECTS OF ESTROGEN ON SUBSTANCE P mRNA LEVELS IN THE RAT ANTERIOR PITUITARY AND HYPOTHALAMUS. E.R. Brown, R.E. Harlan, & J.E. Krause. Dept. of Anatomy & Neurobiology, Washington University Medical School, St. Louis, MO 63110; Dept. of Anatomy, Tulane University School of Medicine, New Orleans, LA 70112

Gonadal steroids alter substance P (SP) peptide levels in the rat anterior pituitary (AP): Estrogen decreases and androgen increases AP SP in male and female rats. In order to determine whether these steroid effects occur at the level of peptide synthesis, we analyzed preprotachykinin (PPT) mRNA levels in individual AP glands, as well as in hypothalami, from intact rats, as well as castrated males (CAST) and ovariectomized females (OX) which were injected daily with either estradiol benzoate (EB; females), testosterone propionate (TP; males), or oil vehicle, for 10 days following surgery. A nuclease protection assay was used to compare levels of the SP-encoding PPT mRNAs (α, β, and γ) in the different treatment groups, and β-actin mRNA, measured by northern analysis, was used as a control. OX resulted in a 5-fold increase over no-surgery control levels, and EB replacement caused a 7-fold reduction below levels in OX rats, in AP β- and γ- PPT mRNAs. On the other hand, in the hypothalamus EB caused a significant increase in all three PPT mRNAs over OX levels. CAST resulted in a decrease, and TP replacement caused an increase back to control levels, in AP β- and γ- PPT mRNAs; this effect was not significant, however, perhaps due to TP aromatization to estrogen. There were no differences in SP-encoding mRNAs in male hypothalami between any of the groups. The EB-induced decrease in these mRNAs in the AP indicates that the estrogen effect on SP levels is at least in part due to changes in peptide synthesis. Furthermore, this regulation is extended to the hypothalamus, where estrogen alters SP-encoding mRNA levels in a direction opposite to that in the AP. These results support the notion that AP and hypothalamic SP is involved in the regulation of AP function.

TRANSMITTER UPTAKE, STORAGE, SECRETION AND METABOLISM III

479.1

RECONSTITUTION OF THE ATP-DEPENDENT GLUTAMATE UPTAKE SYSTEM INTO LIPOSOMES. M. Carlson*, P. Kish* and T. Ueda. Univ. of Michigan Mental Health Res. Inst., Ann Arbor, MI 48109.

We have previously provided evidence for ATP-dependent glutamate uptake into synaptic vesicles, supporting the neurotransmitter role of glutamate. Based upon the unique properties of the vesicular uptake system, we have proposed that the vesicular glutamate translocator plays a crucial role in selecting glutamate for neurotransmission. It is proposed that the vesicular uptake components consist of a glutamate translocator and a proton-pump Mg-ATPase complex. In this study, we have solubilized the vesicular uptake components from brain synaptic vesicles with sodium cholate in the presence of phospholipids and reconstituted the components into phospholipid vesicles (liposomes) by removing the detergent by gel filtration. We demonstrate that the properties of the reconstituted glutamate uptake system are highly similar to those observed in the intact synaptic vesicles, namely ATP-dependency, K_m for glutamate, stimulation by low millimolar concentrations of chloride, sensitivity to electrochemical proton gradient dissipators, and specificity for L-glutamate. The specific uptake activity of the reconstituted system is about 70% higher than that of the native vesicular system. The reconstituted glutamate uptake assay described here represents an initial step toward the separation and purification of the functional proton-pump ATPase complex & the glutamate translocator. (Supported by NSF Grant BNS 8509679.)

479.2

INSULIN-LIKE GROWTH FACTOR-I (IGF-I) ENHANCES CATECHOLAMINE SECRETION FROM BOVINE CHROMAFFIN CELLS. M.K. Dahmer* and R.L. Perlman. Depts. of Peds. and Pharmacol. and Physiol. Sci. and Kennedy Mental Retard. Res. Cent., University of Chicago, Chicago, IL 60637.

We have recently reported that bovine chromaffin cells contain IGF-I receptors (M.K. Dahmer and R.L. Perlman, J. Neurochem. 51, 321-323, 1988). We have now studied the effects of IGF-I on catecholamine secretion from these cells. Chromaffin cell cultures were maintained in serum-free medium in the presence or absence of IGF-I (10 nM) and then incubated for 10 min in medium containing 55 mM K^+; catecholamines released from the cells were assayed by liquid chromatography with electrochemical detection. IGF-I did not affect the survival or the catecholamine content of the cells. Cells that were cultured for 4 days in the presence of IGF-I, however, exhibited a 2-3 fold increase in high K^+-evoked catecholamine secretion. The enhancement of catecholamine secretion was dependent on the IGF-I concentration ($EC_{50} \cong 3$ nM). Insulin also enhanced catecholamine secretion, but was much less potent than IGF-I; thus, the action of IGF-I is probably mediated by IGF-I receptors. IGF-I also enhanced the secretion of catecholamines elicited by ionomycin (3 μM), suggesting that it acts at a step distal to Ca^{2+} entry. IGF-I appears to be an important regulator of chromaffin cell function. (Supported by NIH grants HD04583, HL07455 and HL29025).

479.3

CHOLINERGIC SYNAPTIC VESICLES CONTAIN TWO ATPASE ACTIVITIES. S.M. Parsons, S.K. Yamagata*, and K. Noremberg. Dept. of Chemistry, University of California, Santa Barbara, California 93106.

A glycoprotein MgATPase has been purified from Torpedo californica electric organ synaptic vesicles and shown to have a native-detergent solubilized M_r of 210,000 ± 9000 composed of 110, 104, 98, and 69 kDal subunits. This ATPase is inhibited by vanadate (K_i 10μM), and it accounts for about 2/3 of the total activity in the vesicles. Acetylcholine active transport is not inhibited by vanadate; rather, it is stimulated by 25 percent. Vanadate-insensitive ATPase activity in the vesicles is inhibited by N-ethylmaleimide (K_i 8 μM) as is acetylcholine active transport. This second activity presumably is due to the vacuolar-type ATPase generally postulated to pump protons into most secretory vesicles. The glycoprotein ATPase purified by us forms a phosphoenzyme intermediate with [γ-^{32}P]ATP, but the ATPase is ouabain insensitive and not stimulated by Ca^{2+}. Other properties are being investigated.

479.4

SODIUM-DEPENDENT UPTAKE OF NUCLEOSIDES BY DISSOCIATED BRAIN CELLS FROM RAT. J.D. Geiger. Dept. of Pharmacol., Univ. of Manitoba, Winnipeg, MB. R3E OW3.

Nucleoside transport was studied using a mixed population of dissociated brain cells from adult rat. Accumulation of [^{3}H]adenosine during brief (15 sec) incubation periods was significantly enhanced by the presence of 110 mM sodium. This occurred at substrate concentrations that ranged from 0.25 to 100 uM. Kinetically, the rapid accumulation of [^{3}H]adenosine was best described by a two-component system. In the presence of sodium, the K_T and Vmax values for the high affinity component were 0.9 uM and 8.9 pmoles/mg protein/15 sec, and for the low affinity component were 313 uM and 3428 pmoles/mg protein/15 sec, respectively. In the absence of Na^+, the $K_{T(high)}$ value was significantly higher; 1.8 uM. [^{3}H]Uridine accumulation was best described kinetically by a one-component system that in the presence of Na^+ had K_T and Vmax values of 1.0 mM and 2.6 nmoles/mg protein/15 sec, respectively. As with [^{3}H]adenosine, in the absence of Na^+, the K_T value was significantly higher; 1.8 mM. Sodium-dependent transport of [^{3}H]adenosine was inhibitable by ouabain and 2,4-dinitrophenol. Nitrobenzylthioinosine demonstrated high affinity and selectivity in blocking the sodium component. Thus, high affinity sodium-dependent nucleoside systems, in addition to facilitated diffusion systems, exist on brain cells from adult rats.

479.5

LOCALIZATION OF THE DOPAMINE UPTAKE SITE IN RAT BRAIN USING [^{3}H]-BTCP. J.K.Wamsley, M.Hunt* and F.Filloux. Dept. of Psychiatry, Univ. of Utah Sch. of Med., S.L.C., UT. 84132.

To date, a number of tritiated ligands have been used for autoradiographic labeling of dopamine(DA) uptake sites in the brain. These include [^{3}H]-mazindol, [^{3}H]-nomifensine, [^{3}H]-methylphenidate and [^{3}H]-GBR12935. None have proven satisfactory thus far. ((Benzo(b)thiophenyl-2)-1-cyclohexyl)N-piperidine (GK-13 or BTCP) has been shown to be a potent inhibitor of DA uptake whose affinity for noradrenergic (NE) sites is substantially less, (Chicheportiche et. al.). We have found [^{3}H]-BTCP binding to 10um coronal sections of rat forebrain to be both reversible and saturable and of comparatively high affinity ($K_D \approx$10 - 15nM). The highest specific binding was achieved using 50mM Tris buffer (pH=7.0) which also contained 120mM NaCl. Under these conditions, nonspecific binding was ≤ 45%. Preliminary autoradiograms demonstrate that specific binding in the forebrain is concentrated in the basal ganglia and olfactory tubercle, while little or no specific binding is present in the cerebral cortex. Specific binding is potently displaced by GBR12909, a selective DA uptake inhibitor. No greater degree of displacement is achieved by mazindol, an agent with greater affinity for the NE sites than for DA uptake sites. These data suggest that [^{3}H]-BTCP should be very useful for autoradiographic studies of the dopamine transport complex.

479.6

"SUBSTANCE M," AN IMIPRAMINE BINDING SITE LIGAND FROM HUMAN URINE. K.G. Walton, T. Hauser*, T. McCorkle*, C. Maclean*, D. Fleshman* and R.K. Wallace. Departments of Chemistry and Physiology, Maharishi International University, Fairfield, IA 52556.

Imipramine and other tricyclic antidepressants inhibit the active uptake of serotonin into neurons and blood platelets. This effect involves high-affinity binding of imipramine to a portion of the uptake site where the inhibitor appears to act allosterically. A reduced number of imipramine binding sites in a subset of depressed patients may reflect differences in serotonergic tone between these patients and normal subjects. The possibility that an unidentified endogenous ligand normally regulates serotonin uptake at this site and may play a modulatory role in the serotonin system is under investigation in our laboratory. We have previously reported an inhibition of imipramine binding by extracts of human urine. This inhibitory activity has now been further purified using TLC on silica plates with a mobile phase of acetone / methylene chloride / water / ammonia (80 / 20 / 2 / 0.2). The activity was first concentrated from the urine using a reversed phase C_{18} extraction column (Baker-10 SPE), eluting with acetonitrile. In a typical urine sample, 50% inhibition of [^{3}H]imipramine binding was achieved with extract from 0.1ml of urine (assay volume = 0.5 ml). Eluates from 3 adjacent regions of the TLC plate had inhibitory activity. Reverse phase HPLC of each of these eluates shows 1-3 major and a small number of minor uv-absorbing species. Complete purification and identification of the active ligand(s) seems near.

479.7

COORDINATE STIMULATED RELEASE OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) AND SUBSTANCE P (SP) FROM DISSOCIATED CULTURES OF NEONATAL RAT VAGAL SENSORY NEURONS. D.B. MacLean, Div. of Endocrinology, Brown University, Rhode Island Hospital, Providence, RI 02902

These studies sought to determine whether in cultures of vagal sensory neurons the stimulated release of two neuropeptides, SP and CGRP, is differentially regulated, as previously demonstrated regarding their content. Jugular-nodose ganglia from neonatal rats were enzyme dispersed and co-cultured with rat atriacytes. Neuropeptide content was measured by RIA in release media (KRB, 1% FBS) from 15-20 min epochs between 2-4 weeks in culture. Both SP and CGRP release were stimulated by capsaicin. 34 mM K+-supplemented KRB stimulated SP and CGRP release 6-10x above basal levels, e.g. SP, 23±4 pg and 168±20 pg/well and CGRP 91±27 pg and 702±202 pg/well, basal vs stimulated, x± SD. Bradykinin (BK), maximal effect at 10 nM, resulted in 3-4x basal release e.g. SP, 64±13 pg and CGRP 290±49 pg/well, but did not further enhance K+ release. Histamine did not raise basal or BK-induced release. Serotonin (0.5 uM) weakly stimulated basal release and enhanced BK-evoked release e.g. CGRP 180±55 vs 473±223 pg, BK vs BK + serotonin, P<.01. Forskolin (10 uM) raised both SP and CGRP media content to 2.5-3.5x basal levels; aminophylline did not enhance basal or BK stimulated release. Conclusion: Both CGRP and SP are released from vagal sensory neurons in response to similar stimuli.

479.8

THE DISTRIBUTION OF HIGH AFFINITY UPTAKE SITES FOR [^{3}H] GABA IN THE HUMAN MYENTERIC PLEXUS. A. Krantis*, A. Khalil and C. Krause, Digestive Diseases Research Group, Dept. of Physiology and Div. of Gastroenterology, Univ. of Ottawa, Ottawa, K1H 8M5, Canada.

GABA is a transmitter of myenteric neurons in the guinea-pig, and is proposed to be an enteric neurotransmitter in the rat, cat and human, where GABA and its metabolic enzymes have been localized in neural elements of the intestine wall (Tanaka 1985, Life Sciences, 37: 2221-2235). However, the disposition of GABAergic neurones in the human enteric nervous system is unknown. Therefore we sought to determine the occurrence and disposition of high affinity uptake sites for [^{3}]GABA (characteristic of GABA nerve cells) in the human large intestine. Autoradiography (after Krantis et al. 1986, Neuroscience 17(4): 1243-1255) was performed on paraffin sections (12-20 μm) of various segments of colon taken at surgery and incubated with 50 Ci/mmole (Amersham) 2,3 [^{3}]GABA, 5.10^{-9} to 10^{-8}M, in the absence or presence of specific inhibitors of high affinity GABA uptake. Radiolabelled GABA was accumulated by a sub-population of myenteric neurones in all segments examined. However, few labelled processes could be seen. These results show that GABA is transported into human myenteric neurones by a high affinity system.

Funded by the Medical Research Council of Canada.

479.9

QUINOLINIC ACID FORMATION IN RAT BRAIN IN VIVO AND IN VITRO: EFFECTS OF STRIATAL LESIONS. C. Speciale, U. Ungerstedt* and R. Schwarcz. Maryland Psych. Res. Ctr., Baltimore, MD 21228.

In view of the potential role of the excitotoxic brain metabolite quinolinic acid (QUIN) in neurodegeneration, we have begun to examine mechanisms controlling its synthesis in the brain. Both in vivo (using brain microdialysis in awake rats) and in vitro (in brain slices), local pretreatment with QUIN's putative bioprecursors tryptophan and kynurenine (1 mM) failed to yield QUIN. In contrast, QUIN production was demonstrated after tissue exposure to as little as 10 µM 3-hydroxyanthranilic acid (3HANA). Since 3HANA-oxygenase activity is greatly increased in lesioned brain (Brain Res., 436: 18, 1987), the ibotenate (40 µg)-injected rat striatum was examined for its ability to produce QUIN from 3HANA. One week after surgery, striata of lesioned (IBO) or saline-treated (SAL) rats were perfused with 30 µM 3HANA. Extracellular QUIN, collected hourly, increased more rapidly in IBO (75±10 vs. 28±6 pmoles/50 µl during the 2nd hour; $p<0.01$) than in SAL rats. After in vitro exposure of striatal slices to 30 µM 3HANA (30 min, 37°C), 135±9 vs. 49±2 pmoles QUIN/mg protein, respectively, were detected in the medium after incubation with slices from IBO and SAL rats ($p<0.01$). Increased extracellular levels of QUIN in lesioned tissue may play a role in neurodegenerative processes. (Supported by a Fogarty Fellowship (to C.S) and USPHS grant NS 16102).

479.10

KYNURENINE AMINOTRANSFERASE IN HUMAN BRAIN. M. Nakamura, E. Okuno, [1]W.O. Whetsell Jr. and R. Schwarcz. Maryland Psych. Res. Ctr., Baltimore, MD 21228 and [1]Dept. Pathol., Vanderbilt Univ. School Med., Nashville, TN 37232.

The cerebral biosynthesis of the putative neuroprotectant kynurenic acid (KYNA), a constituent of human brain, has as yet not been elucidated. In rat tissue, four enzymes have been identified which can transaminate L-kynurenine to KYNA, and one transaminase has been described in the human liver. Using a conventional spectrophotometric assay (Biochem. J., 189: 581, 1980), we found kynurenine transaminase (KYNT) activity in thawed human brain tissue homogenate. KYNT activity was dependent on α-ketoglutarate (αKG) and pyruvate (PYR) as aminoacceptors. Product formation was confirmed by HPLC analysis. Linearity of the enzymatic reaction was ascertained up to 2 hours of incubation and up to 600 µg protein/assay tube. In the course of our efforts to purify KYNT from human brain, αKG and PYR were used as aminoacceptors in parallel at every purification step. Differences existed with regard to both the yield of activity and specific activity when the results using αKG and PYR were compared, indicating the presence of at least two KYNT proteins in the human brain. Efforts are now under way to further purify and characterize the two enzymes, and to examine their respective roles in the anabolism of neuroactive KYNA.

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479.11

SYNTHESIS OF KYNURENIC ACID FROM ITS BIOPRECURSOR L-KYNURENINE IN RAT BRAIN IN VITRO AND IN VIVO. W.A. Turski, J.B.P. Gramsbergen, C. Speciale, U. Ungerstedt* and R. Schwarcz. Md. Psych. Res. Ctr., Baltimore, MD 21228.

The neuroprotective broad spectrum excitatory amino acid antagonist kynurenic acid (KYNA) has recently been identified in mammalian brain. In the present study, we have examined the synthesis and liberation of KYNA from rat brain in vitro (slices) and in vivo (microdialysis) after exposure of the tissue to L-kynurenine (KYN). After incubation, brain slices produced KYNA in a tissue-, dose (KYN)-, time-, temperature-, oxygen- and glucose-dependent fashion. Under standard conditions (2 h incubation at 37°C using 50 µM KYN in regular, oxygenated Krebs-Ringer buffer), cortical slices produced about 100 pmol KYNA per mg protein. De novo synthesized KYNA was rapidly liberated into the incubation medium, and its production could be blocked by the non-selective transaminase inhibitor, aminooxyacetic acid (IC_{50}: 25 µM). KYNA production and liberation did not depend on the presence of Ca^{2+}-ions and remained unchanged in high Mg^{2+} (20 mM) medium. Experiments with slices derived from ibotenate lesioned striata (40 µg/2 µl; 7 days after surgery) suggest a predominantly glial localization of KYNA production.

After in vivo perfusion with 500 µM KYN, KYNA was detectable in 30 min fractions of striatal dialysates, reaching plateau concentrations after about 3 h.

Supported by USPHS grants NS 16102 and NS 20509.

479.12

NEURONAL ACTIVITY AFFECTS KYNURENIC ACID PRODUCTION IN RAT BRAIN SLICES. J.B.P. Gramsbergen, W.A. Turski and R. Schwarcz. Maryland Psych. Res. Ctr., Baltimore, MD 21228.

The endogenous excitatory amino acid antagonist and brain metabolite kynurenic acid (KYNA) may play a role in the prevention of excitotoxic brain damage.

KYNA can be produced by brain slices in vitro upon exposure to its bioprecursor L-kynurenine (KYN; cf. Turski et al., this meeting). The effect of different experimental conditions was assessed by measurement of liberated KYNA in the incubation medium. Blockade of neuronal activity with 1 µM TTX or by incubation in Na^+-free medium gave rise to higher concentrations of KYNA (+23% and 31%, respectively; $p<0.05$), whereas depolarization of the slices by K^+ (50 mM) or veratridine (5 µM) resulted in a substantial decrease (-38% and -68%, respectively; $p<0.001$) of KYNA content in the medium. The effects of both veratridine and high K^+ were blocked in Na^+-free incubation medium. Furthermore, the effect of veratridine was inhibited by TTX and the effect of high K^+ could be attenuated in Ca^{2+}-free medium and was blocked completely by 20 mM Mg^{2+}.

Both high K^+ and veratridine were not effective in experiments performed with slices derived from ibotenate lesioned (40 µg/2 µl; 7 days postsurgery) striata. Taken together, our data show that neuronal activity (and possibly a releasable factor), rather than direct depolarization of glial cells, affects the (glial) production of KYNA.

Supported by USPHS grants NS 16102 and NS 20509.

EXCITATORY AMINO ACIDS X

480.1

ANTAGONIST PHARMACOLOGY OF EXCITATORY AMINO ACID RECEPTORS EXPRESSED IN XENOPUS OOCYTES. T.A. Verdoorn and R. Dingledine. Dept. Pharmacology and Curr. Neurobiol., Univ. North Carolina, Chapel Hill, NC 27599.

Quantitative pharmacological studies of neuronal EAA receptors were done in Xenopus oocytes injected with rat brain mRNA. Inward currents induced by NMDA and kainate were both antagonized by CNQX (FG 9065). The properties of antagonism were different for NMDA and kainate receptors, however. CNQX was a competitive antagonist of the receptor mediating the kainate current as determined by Schild analysis (pA_2 = 6.53 ± 0.02, slope = 1.02 ± 0.02, n=19) up to a dose ratio of approximately 100. The block of NMDA receptors by CNQX was non-competitive since 6 µM and 15 µM CNQX reduced the maximum NMDA current by 49 ± 8% (n=3) and 69 ± 6% (n=3) respectively, and 15 µM CNQX increased the NMDA EC_{50} by no more than 2-fold. CNQX had little or no effect on the oscillating chloride current induced by quisqualate and caused no detectable ionic current by itself. D-APV antagonized currents induced by NMDA and L-aspartate in an apparently competitive manner up to D-APV concentrations of at least 100 µM (approximate dose ratio = 100). The pA_2 of D-APV was 5.85 ± 0.04 (slope = 1.03 ± 0.03, n=9) against NMDA currents and 5.86 (n=2) against L-aspartate currents. D-APV alone caused no ionic current. These data indicate NMDA and L-aspartate currents may be mediated by the same receptor. The non-competitive block of NMDA currents by CNQX further distinguishes NMDA receptors from kainate receptors.

480.2

EXPRESSION OF THE N-METHYL-D-ASPARTATE/PCP RECEPTOR IN XENOPUS OOCYTES INJECTED WITH mRNA FROM NCB-20 CELLS. L. Kushner*, J. Lerma*, M.V.L.Bennett and R.S. Zukin Albert Einstein College of Medicine., Bronx, NY 10461

The N-methyl-D-aspartate (NMDA) receptor complex is a ligand-gated cation channel which contains regulatory binding sites for Mg^{2+}, Zn^{2+} and glycine. Recent evidence suggests that this receptor complex also contains the phencyclidine (PCP) receptor which mediates the psychotomimetic effects of PCP derivatives, σ opioids and the dioxalanes, and that PCP acts as a blocker of the NMDA gated channel. The mouse neuroblastoma-Chinese hamster brain hybrid cell line NCB-20 has a PCP receptor similar to the rat brain receptor based on quantitative receptor assays under equilibrium binding conditions. This site was labelled by the potent phencyclidine derivative N-[1-(2-thienyl)cyclohexyl]piperidine (TCP) with a binding affinity (K_d) of 335 nM and a receptor density (B_{max}) of 9264 fmol/mg protein. In order to investigate the molecular basis of the NMDA/PCP receptor of NCB-20 cells we looked for its expression in Xenopus oocytes injected with NCB-20 cell poly(A^+)RNA (50 ng/cell) Oocytes were voltage clamped and perfused with Mg^{2+}-free amphibian Ringer's solution. Drugs were bath-applied. At a holding potential of -60 mV, NMDA (EC_{50} = 20 µM, with 10 µM glycine) evoked a partially desensitizing inward current that was potentiated by glycine (EC_{50} = 0.1 µM) and inhbited by the competitive antagonist D-(-)-amino-5- phosphonovaleric acid (IC_{50} = 3 µM). NMDA currents were blocked by PCP (0.1 µM) and MK-801 (0.1 µM) in a use dependent manner. There was little or no response to quisqualate (10 µM), kainate (500 µM) and GABA (100 µM). These data indicate that the NCB-20 cells contain mRNA which encodes a NMDA/PCP receptor like that of neurons.

480.3

NMDA, KAINATE AND QUISQUALATE RECEPTORS OF RAT BRAIN EXPRESSED IN XENOPUS OOCYTES: SUMMATION EXPERIMENTS INDICATE THAT EACH GATES SEPARATE CHANNELS. J. Lerma*, L. Kushner*, M.V.L. Bennett, and R.S. Zukin (SPON: E. Masurovsky) Albert Einstein College of Medicine, Bronx, NY 10461.

Rat brain mRNA injected into Xenopus oocytes leads to responsiveness to the glutamate agonists NMDA (N), kainate (K) and quisqualate (Q) (Kushner et al., PNAS 85: 3250, 1988). Sharing of channels by receptors for these agonists has been suggested (Jahr and Stevens, Nature 325: 522, 1987). However, the present study of voltage-clamped oocytes indicate independence: 1) The Hill coefficients differ (c.1.0, 1.5 and 1.9 for N, K and Q, respectively). 2) PCP receptor ligands are channel blockers selective for NMDA receptors. When NMDA channels are blocked by trapped PCP, K and Q currents are unaffected. 3) NMDA and Q currents summate linearly. 4) NMDA and K currents summate with a 10-20% deficit. Part of the deficit is because NMDA is a weak antagonist at K receptors; NMDA in the presence of a sufficient concentration of APV, PCP or Mg to block NMDA responses reduces K currents slightly, whereas APV alone has a lesser effect and PCP or Mg alone has no effect. The remainder of the deficit is ascribable to weak antagonist action of K at NMDA receptors. 5) Q currents are usually much smaller than K currents. Saturating Q slightly facilitates responses to low concentrations of K and reduces responses to high concentrations of K. Plausibly, Q is a partial agonist at K receptors, such that K receptors occupied by 2Q are inactive, QK are partially active and 2K are maximally active. Although specificities of the available drugs are not absolute, these data support the classical concept that NMDA, K and Q act with highest affinity at different binding sites each gating its own channel.

480.4

COMPETITIVE AND NON-COMPETITIVE BLOCK OF NMDA/PCP RECEPTORS EXPRESSED IN XENOPUS OOCYTES. M.V.L Bennett, J. Lerma*, L. Kushner* and R.S. Zukin. Albert Einstein College of Medicine, Bronx, NY 10461

NMDA receptors were expressed in Xenopus oocytes following injection of rat brain mRNA (Kushner et al. PNAS 85: 3205, '88). Glycine is required for responses to bath-applied NMDA. Responses to varying NMDA concentrations (with 10 μM glycine, steady state, partially desensitized) have a Hill coefficient, n=1 and K_d=16 μM. I_{max} increases with glycine concentration, but n and K_d are unaffected, consistent with allosteric potentiation. APV (EC_{50}=3 μM) is a competitive blocker; K_d is shifted to higher concentrations with little effect on n or I_{max}. Block by PCP is non-competitive; I_{max} is reduced without change in K_d or n. PCP appears to be a channel blocker, and block and unblock exhibit use dependence. PCP alone blocks very slowly; block develops rapidly in the presence of NMDA. Rate of onset of block increases with agonist concentrations (when channel open probability is higher), but degree of block depends on PCP and not NMDA concentration (indicating that PCP enters and leaves channels primarily if not exclusively when they are open). Removal of agonist leaves the channel blocked. Recovery is slow in the absence of applied agonist and is speeded by reapplication. Block is voltage dependent in that at more inside positive voltages recovery (in the presence of agonist) is faster. Mg^{2+} shows similar non-competitive and voltage dependent block (ED_{50}=10 μM), but on and off times are too rapid to demonstrate trapping in the channel. Block by Zn^{2+} (EC_{50}=10 μM) is non-competitive but less voltage dependent. These results show further characteristics of the NMDA/PCP receptor expressed in oocytes and strengthen the view that PCP is an open channel blocker.

480.5

7-CHLOROKYNURENIC ACID: A SELECTIVE ANTAGONIST AT THE GLYCINE MODULATORY SITE OF THE NMDA RECEPTOR COMPLEX. A.C. Foster, J.A. Kemp*, P.D. Leeson*, R. Tridgett*, T. Priestley* and G.N. Woodruff*. Merck Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex, U.K.

Glycine has been shown to markedly potentiate responses in cultured cerebral neurones mediated by N-methyl-D-aspartate (NMDA) receptors (Johnson and Ascher, Nature, 325: 529, 1987). Electrophysiological experiments using isolated outside-out membrane patches (ibid) and radioligand binding studies (Wong et al, Eur. J. Pharmacol. 142: 487, 1987) indicate an allosteric interaction through a strychnine-insensitive glycine recognition site which is part of the NMDA receptor complex. Here we present evidence that 7-chlorokynurenic acid (7-Cl KYNA) inhibits NMDA responses by a selective antagonism of glycine at its modulatory site. In rat cortical slices 7-Cl KYNA (10-100μM) non-competitively inhibited NMDA responses and this effect could be reversed by the addition of glycine (100μM) or D-serine (100μM). Radioligand binding experiments showed that 7-Cl KYNA had a much higher affinity for the strychnine-insensitive [^{3}H]glycine binding site (IC_{50} 0.56μM) than for the NMDA (IC_{50} 169μM), quisqualate (IC_{50} 153μM) or kainate (IC_{50} > 1000μM) recognition sites. Whole cell patch clamp recordings from rat cortical neurones in culture also indicated that the inhibition of NMDA responses by 7-Cl KYNA could be reversed by glycine and in addition basal NMDA responses were abolished, suggesting a negative modulatory effect of 7-Cl KYNA at the glycine site. These findings indicate that the glycine modulatory site of the NMDA receptor is functional in intact tissue and that 7-Cl KYNA may be a useful tool for the study of its involvement in CNS function.

480.6

1-HYDROXY-3-AMINOPYRROLIDONE-2 (HA-966) AND KYNURENATE ANTAGONISM OF N-METHYL-D-ASPARTATE (NMDA) RECEPTOR MEDIATED EVENTS IN SLICES OF RAT NEOCORTEX IS REVERSED BY GLYCINE. E.J. Fletcher* and D. Lodge* (SPON: M. Duchen). Dept. Basic Vet. Sci., Royal Vet. Coll, London NW1, U.K.

Glycine potentiated responses evoked by NMDA in patch clamp studies of Johnson & Ascher (Nature, 325:529, 1987), via a strychnine-insensitive site near the NMDA receptor.

On rat cortical wedges, however, we found that glycine (10uM-1mM) did not enhance depolarisations evoked by NMDA. But, when NMDA responses were reduced by either kynurenate or HA-966, glycine reversed the antagonism of NMDA but not that of quisqualate. Thus, 200uM kynurenate reduced NMDA responses to 59 + 2% of control whilst in the presence of 100uM glycine this was only 32 + 5%. 200uM HA-966 alone reduced the response to NMDA by 32 + 0.6% but in the presence of 100uM glycine by only 8 + 4%. NMDA antagonism produced by magnesium, aminophosphonovalerate (AP5), ketamine and dextrorphan was not reversed by glycine.

Spontaneous synaptic activity in cortical wedges bathed in magnesium-free medium was reduced by magnesium, AP5 and kynurenate, but only the latter was reversed by glycine.

These results suggest that glycine tonically facilitates synaptic responses mediated by NMDA receptors and that HA-966 may be useful in characterising this novel modulatory site on the NMDA receptor channel complex.

Supported by the Wellcome Trust.

480.7

MULTIPLE CLASSES OF EXCITATORY AMINO ACID RECEPTORS ON CULTURED EMBRYONIC AMPHIBIAN SPINAL NEURONS. S.B. Sands and M.E. Barish, Department of Physiology and Biophysics, University of California, Irvine, CA 92717.

Activation of excitatory amino acid (EAA) receptors has been implicated in mechanisms of CNS synaptic transmission including long term synaptic potentiation. EAA receptors may also play a role in the structural development of the CNS. To address this last question we are investigating the development and characteristics of EAA receptors in cultured embryonic *Xenopus* spinal neurons using the patch clamp technique. The internal solution used for whole cell and outside-out patch recording is primarily CsCl. Drugs are transiently applied to the cell under study with a perfusable extracellular pipette.

Responses to glutamate (GLU) and three GLU agonists, kainate, quisqualate and N-methyl-D-aspartate (NMDA), which define different GLU receptor classes, are present on ~60% of neurons examined within 12 hours of dissociation from late neural plate-stage embryos. A consistent association of GLU with glycine (GLY) responses suggests that these cells are motoneurons. The threshold for GLU response is <1 μM; half maximal concentration is ~20 μM. The GLU response is potentiated by low (<2 μM) concentrations of GLY, and is stereospecific for the L isomer. Responses to application of NMDA (10-100 μM) in Mg-free external solution are rarely observed, but can be exposed by simultaneous application with GLY. Potentiation of NMDA responses by GLY occurs with a half maximal concentration of <1 μM.

The effects of activation of these receptors on neurite outgrowth is currently under investigation.

480.8

DOES QUISQUALATE ACTIVATE A FAST TRANSIENT CURRENT? Cha-Min Tang* Marc Dichter and Martin Morad*(SPON:B Twarog) Dept. of Physiol. and Neurol., U of Pa., Phila.,Pa. 19104

There is no general agreement whether the quisqualate (quis) activated current desensitizes (JP 399;224, NSL 63:225). In cultured hippocampal neurons we find that the speed of drug application and the concentration tested can account for the reported discrepancy. We observe both a fast transient and a non-desensitizing current upon rapid step application fo quis in every neuron tested, supporting the report of Grantyn et al (BR 420:182). The dose response suggests two quis are required to activate each channel mediating the persistent current and activation occurs at low concentrations (half max. at 0.2 uM). The transient current is activated only above 1 uM (K_D~10-20uM). The transient current, however, shows steady state desensitization at the low concentrations that activate the persistent current. The desensitization follows a single exponential (τ~30-40 msec) and is voltage insensitive. Thus in order to observe the transient current quis has to be increased from below the inactivatingto the activating concentrations within 2-3 τ's (<100msec). In about half of the neurons the persistent current showsprominent suppression at quis concentrations above 1 uM (negative cooperativity). It is not known if these current components are mediated by a single receptor/channel complex with multiple agonist binding and allosteric interactions or separate channels.

480.9

PHYSIOLOGICAL REGULATION OF GLUTAMATE RECEPTOR FUNCTION BY DESENSITIZATION. L.O. Trussell & G.D. Fischbach
Dept. Anat. & Neurobiol., Washington U. Sch. of Med., St. Louis MO 63117.

Responses of voltage-clamped cultured chick spinal neurons to prolonged application of glutamate fade with a time course dependent on the receptor type and the method of application. Rapid application of 1 mM glutamate to outside-out membrane patches show that G_2 (APV resistant) currents fade with a $t_{1/2}$ of 5 ms. The concentration dependence of desensitization was studied by whole cell recording. Bath application of 2-100 μM glutamate (with 2-APV) reduced the responses to ionophoretic glutamate pulses applied at receptor hot spots with an ID_{50} of 8 μM. This value is in the range of concentrations that bathe neurons in vivo and in vitro (2-15 μM). Significant reduction was observed with glutamate concentrations that produced little or no receptor activation. Indeed, superfusing cells with fresh, glutamate-free solutions often increased the size of ionophoretic glutamate responses. Evoked and spontaneous excitatory synaptic currents were reduced in amplitude by 48±17% (± S.D., N=4 cells) following application of 10 μM glutamate. These data suggest that receptor desensitization by normal concentrations of glutamate modulate the efficacy of central synaptic transmission. This work was supported by NS #18458 and the ALS Association.

480.10

CHARACTERIZATION OF A RAPIDLY DESENSITIZING GLUTAMATE CURRENT IN CULTURED POSTNATAL HIPPOCAMPAL PYRAMIDAL NEURONS **L.L. Thio, D.B. Clifford, and C.F. Zorumski, Washington University School of Medicine, Departments of Psychiatry and Neurology, St. Louis, MO 63110**

Rapid pressure applications of glutamate evoke a rapidly activating and rapidly decaying inward current in >95% of the cultured neonatal rat hippocampal pyramidal neurons studied with the whole cell patch clamp technique. The decay occurs despite the continued application of glutamate, and is followed by a steady inward current. The fast, transient current is not the product of an electrical, pressure, or enzymatic artifact. While NMDA, kainate, domoate, ibotenate, and D or L-homocysteate do not evoke this rapidly desensitizing current, other structural analogs of glutamate such as quisqualate, AMPA, willardiine, and bromowillardiine do. The quisqualate current has been examined in further detail. Both the transient and steady-state phases have linear IV relationships with reversal potentials near 0mV. The fast, transient and steady-state currents are activated by quisqualate in a dose-dependent manner with K_{ap}'s of 50μM and 3μM, respectively. The fast, transient current evoked by 100μM quisqualate reaches half its peak value in 20ms and decays exponentially to 26% of its peak value with a time constant of 81 ms. The current recovers completely in less than 4s. The percentage of desensitization, the time constant of decay, and the time of recovery are identical at +50mV and -50mV.

480.11

QUISQUALIC ACID INFLUENCES KAINATE-INDUCED RESPONSES IN CULTURED CEREBELLAR GRANULE CELLS: BIOCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES. V. Gallo+, S.G. Cull-Candy* and M.M. Usowicz*. +Neurobiology Section, Pathophysiology Laboratory, Istituto Superiore di Sanita', 00161 Rome, Italy, and MRC Receptor Mechanisms Group, Pharmacology Dept., University College, London WC1E 6BT, U.K.

Kainic acid (KA) (20-300μM) and quisqualic acid (QUIS) (5-300μM) stimulated the release of preaccumulated ^{3}H-D-aspartate from rat cultured cerebellar granule cells in a dose-dependent manner. This effect of KA, but not that of QUIS, could be antagonized by kynurenic acid (KYN) (50-200μM) or by 2,3-cis-piperidine dicarboxylic acid (200μM). QUIS (10-50μM) inhibited in a dose-dependent manner the ^{3}H-D-aspartate release induced by 50μM KA. Under these conditions, ^{3}H-D-aspartate release induced by KA+QUIS was not antagonized by KYN. However, simultaneous application of ineffective concentrations of KA (10μM) and QUIS (2-5μM) evoked the release of ^{3}H-D-aspartate, and this release could also be blocked by KYN. Moreover, QUIS (2-50μM) or KYN (50-200μM) antagonized KA-induced cGMP formation: half maximal concentrations were 5μM QUIS and 100μM KYN.

These biochemical experiments were extended by complementary electrophysiological studies. Whole-cell voltage clamp recording from cerebellar granule cells showed that currents generated by 30-100μM KA were reversibly diminished by KYN (100-200μM), both at negative and positive holding potentials. Currents produced by KA were also inhibited by 5μM QUIS. This inhibition by QUIS was greater for currents evoked by 100μM KA, than for the smaller currents produced by 30μM KA. Similar observations have been made in retinal horizontal cells (Ishida and Neyton, 1984, P.N.A.S. 82, 1837). The whole-cell experiments suggest that QUIS influences KA-responses in cerebellar granule cells, possibly acting at KA receptors.

480.12

QUISQUALATE EFFECTS IN CULTURED NEURONS FROM THE RAT SUPERIOR COLLICULUS: A PATCH-CLAMP STUDY. M. Perouansky+ and R. Grantyn (SPON: H. Holländer). MPI, 8033 Martinsried, FRG

Cultured neurons from the visual layers of the rat superior colliculus express at least three different receptors for acidic aminoacids (Perouansky and Grantyn, J. Neurosci. 1988). Quisqualate-(QA)-selective components are resistant to NMDA- and kainate-(KA)-receptor antagonists such as D-2-amino-5-phosphonovaleric acid (APV) and kynurenic acid. A QA-receptor-mediated component can therefore be isolated in the compound response of tectal neurons to exogenous L-glutamate (Glu). Binding to QA-receptors produced up to half of the current elicited with 100 uM Glu.

All collicular neurons from E21/P1 rats responded to QA, its EC_{50} ranging from 0.1 to 0.3 uM. Application of QA modifies the response to other Glu-agonists. Testing QA with various concentrations of KA showed that QA acts as a competitive antagonist of KA. The effect of QA on subsequently elicited $I_{(NMDA)}$ is more complex. The initial transient component of $I_{(NMDA)}$ was suppressed, while the persistent component temporarily recovered from its block by APV. These experiments demonstrate, thus, a modulatory action of QA on NMDA-receptors.

QA had a strong effect on GABA-release, even under the condition that voltage-activated Na and Ca currents were fully blocked. QA-induced depolarization of presynaptic terminals and/or liberation of Ca from intracellular stores by activation of second messenger chains may account for this novel effect of QA.

480.13

ACTIONS OF KYNURERIC ACID ON THE NMDA-ACTIVATED CATIONIC CHANNEL: A PATCH CLAMP STUDY.
M.Bertolino, S.Vicini and E. Costa., (SPON: M. Santi). FGIN, Georgetown Univ., Washington D.C. 20007.

N-D-methyl-aspartic acid (NMDA) activates cationic channels in outside-out patches excised by neonatal rat cortical neurons in primary culture. These channels openings are characterized by a mean channel opening duration of 5-6 msec and conductance of 50 pS . D-2-amino-5-phosphonovalerate (APV), an antagonist of the NMDA recognition site on glutamate receptor reduced dramatically the frequency of these opening observed in the outside-out membrane patches exposed to NMDA. Kynurenic acid, a metabolite of tryptophan, produced as well reduction of these openings. Glycine was shown to potentiates NMDA responses increasing the channel opening frequency. APV and kynurenic acid both, counteract this glycine potentiation. APV action is probably due to competition for glutamate binding sites while kynurenic acid seems to have a non competitive action on this site as shown by whole-cell current dose-response to ionophoretically applied NMDA in presence and absence of kynurenic acid and APV. Supramaximal concentration of glycine are able to partially overcome the kynurenic and not the APV induced reduction of channel opening frequency. The interaction between these compounds will be discussed.

481.1

OLEIC ACID INHIBITS SHAM FEEDING WHEN DUODENALLY INFUSED WHILE TRIOLEIN DOES NOT. D. Greenberg, G.P. Smith, and J. Gibbs. Dept. of Psychiatry, New York Hospital-Cornell Medical Center White Plains, NY 10605.

The specific satiety stimulus provided by ingested fats is unknown. A long-chain triglyceride mixture [Intralipid (Kabi Vitrum Inc., CA)] or a single long chain fatty acid (oleic) elicits satiety when infused intraduodenally in sham feeding rats. We compare here the satiating potency of oleic acid with its triglyceride triolein.

Method: Rats were equipped with gastric cannulas for sham feeding and Silastic catheters for duodenal infusions. After 17h food deprivation, rats were permitted to sham feed liquid food (BioServ 40% v:v). Infusions began 12 min after sham feeding began and lasted 26 min. Infusions were 10 ml of oleic acid (6.5 and 13 kcal), 10 ml of triolein (2.5, 5 ,10 and 20 kcal) or 10 ml of 0.15M NaCl.

Results: Oleic acid infusions significantly reduced sham feeding by %58 (6.5 kcal) or %69.5 (13 kcal) [$F(2,6) = 40.8$, $p<.001$] compared to saline infusion. Triolein failed to significantly reduce sham feeding [maximal %17 (10 kcal); $F(3,13) = 1.91$, $p<.1$].

Conclusions: These results suggest that for long-chain fats, fatty acids are more satiating than are triglycerides.

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481.2

BLOCKADE OF CHOLECYSTOKININ (CCK) SATIETY IN GENETICALLY OBESE ZUCKER RATS. A.J. Strohmayer, D. Greenberg, R. von Heyn*, L. Dornstein* and C. Balkman*, Dept Psychiatry, Cornell Univ Med Coll, Manhasset NY 11030, USA.

The specific CCK receptor antagonist L364,718 was used in the present study to block endogenous CCK satiety in Zucker rats. 6 male fa/fa obese and 6 male Fa/Fa leans were adapted to a 6 hour food deprivation, and IP injections 30 min before a one hour test meal of liquid food. Rats were injected with either L364,718 (0.375 mg/kg) or vehicle. Each rat was tested twice at this dose, the results pooled and significance determined by correlated T-test. Food intake was increased 25% in lean rats following L364,718 (19.6 ml±1.06) compared to vehicle alone (16.1 ml±0.64,T=4.072,p<0.01). Obese Zucker rats did not increase food intake following L364,718 (16.9 ml±1.33) compared to vehicle (15.5 ml±1.55, T=1.493,p>.1). The results suggest that blocked CCK receptors cause over eating in lean rats, while the blockade had no effect on obese rats, suggesting they do not release endogenous CCK. These data support an hypothesis of a failure of CCK satiety in the genetically obese Zucker rat.

481.3

THE EFFECTS OF CCK-8 INFUSION ON INTAKE AND GASTRIC EMPTYING IN NON-OBESE WOMEN. J. Guss*, H.R. Kissileff, R. Pierson* and F.X. Pi-Sunyer*. Obesity Research Center, St. Luke's\Roosevelt Hospital & Columbia University, N.Y., NY 10025.

To test the hypothesis that slowing of gastric emptying mediates the effect of CCK-8 on food intake in women, gastric emptying and intake of a test meal were measured after two doses of CCK-8 (1.125 µg/min .05625 µg/min) and saline given on nonconsecutive days in six women. The infusion began 10 min before the meal and continued for 5 min into the meal. Subjects ate a radiolabeled (technetium-99m-DTPA) soup preload (500 g) 20 min before the test meal. Intake was significantly ($p<.03$, 1-tailed) less on the larger CCK-8 dose (190 g) than on saline (319 g). Although there was no correlation between the amount of soup emptied before the meal and intake of the meal, there was a significant correlation ($r^2 = .93$ high dose CCK, $r^2 = .82$ for low dose CCK-8) between amount eaten in the test meal and amount of soup emptied during the test meal on days when CCK-8 was infused but not on days when saline was infused. Therefore, CCK-8 may faciliate signals of satiety from the stomach, but that stomach fullness per se does not mediate CCK-8's effects on emptying. (Supported by NIH grant #DK36507.)

481.4

COMPARATIVE EFFECTS OF THE CCK ANTAGONIST L364,718 ON FOOD INTAKE AND PANCREATIC EXOCRINE SECRETION IN RATS. R.D. Reidelberger*, M.F. O'Rourke* and T.E. Solomon* (SPON: J. Campbell). Depts. of Physiol. and Med., Univ. Kansas Med. Sch. and V.A. Med. Ctr., Kansas City, MO 64128.

The CCK receptor antagonist L364,718 was used to define the role of CCK in food-stimulated pancreatic secretion and satiety in rats. For pancreatic studies, animals were prepared with gastric, jugular vein, bile-pancreatic duct, and duodenal cannulas. In rats receiving a maximal stimulatory dose of CCK8 (200 pmol/kg-h), L364,718 (0.02, 0.1, 0.5, 1, 2 mg/kg i.v.; Merck Sharp & Dohme) caused dose-related inhibition of amylase output which reached levels > 90% at 0.5 mg/kg and higher. A liquid meal increased amylase output maximally; L364,718 (0.5 mg/kg) abolished the response. In food intake studies, L364,718 (0.01, 0.03, 0.1, 0.3 mg/kg i.p.) caused dose-related reversal of the maximal inhibitory effect of exogenous CCK8 (8 nmol/kg) on liquid food intake; threshold and maximal doses were 0.03 mg/kg and 0.3 mg/kg. L364,718 alone (0.03, 0.1, 0.3, 1 mg/kg) stimulated liquid and solid food intake dose-dependently; threshold dose was 0.1 mg/kg for each diet. Short-term intake (2-3 h) was increased by 16 to 35%; 22-h intake by 7 to 22%. We conclude: 1) L364,718 acts as a long-lasting inhibitor of exogenous CCK8 on pancreatic secretion and food intake; 2) endogenous CCK is important in the physiologic control of food intake and pancreatic exocrine secretion in rats.

481.5

THE EFFECTS OF CHRONIC AND ACUTE TREATMENT WITH THE POTENT PERIPHERAL CHOLECYSTOKININ ANTAGONIST L-364,718 ON FOOD AND WATER INTAKE IN THE RAT. CA Watson*, LH Schneider, ES Corp*, SC Weatherford, R Shindledecker, RB Murphy, GP Smith and J Gibbs. (SPON: SH Ackerman) Dept Psychiatry, NY Hosp-Cornell Med Ctr, White Plains, NY 10605; Dept Chemistry, New York Univ, NY, NY 10003

Cholecystokinin (CCK) released by ingested food may act as a physiological mechanism limiting food intake. To test this hypothesis, we compared the effects of chronic and acute treatments with the potent, highly selective CCK receptor antagonist L-364,718. Rats (male, S-D, 490-550g) were maintained (23 h/d) on a liquid diet under a reversed light/dark cycle. L-364,718 (n=9) or vehicle (n=9) was injected ($i_1p.$) at 1000 h (0.5 $mg\text{-}kg^{-1}$) and at 1400 h (1.0 $mg\text{-}kg^{-1}$). L-364,718 increased food intake both from 1030-1330 h (24%) and over the first 24-h (32%). The magnitude of these increases declined progressively so that by day 7, intake did not differ from controls, and remained so for the rest of the 14-d period. By contrast, 24-h water intake was significantly elevated with respect to controls throughout the experiment. Results from the acute study (n=18) replicated the day 1 results of the chronic study. These findings confirm and extend those of Reidelberger and O'Rourke (1987). They are consistent with an important role for endogenous CCK in the control of food intake. [Supported in part by MH15455 (GPS), MH00149 (GPS), NS24781 (LHS)]

481.6

DOUBLE-LABEL IMMUNOHISTOCHEMICAL LOCALIZATION OF CHOLECYSTOKININ (CCK) AND METHIONIN-ENKEPHALIN (ENK) IN THE RAT NUCLEUS TRACTUS SOLITARIUS (NTS). P.L. Faris*, B.K. Hartman, J.P. Kettinger*, J.N. Howard*, J. Chen[1], A. Nadzan[1] and J.F. McKelvy[1]. (SPON: J. Gottesman) Depts. of Psychiatry and Pathology, Univ. of Minnesota, Mpls, MN 55455 and Abbott Labs, Abbott Park, IL 60064.

Recent attention has focused on the role of the NTS in mediating peptidergic influences on food intake and nociception. CCK and ENK have been shown to have opposite effects on these behavioral parameters although the exact anatomical loci underlying this physiological antagonism is not known in many cases. This study investigated interactions between CCK and ENK immunolabeled neurons in the NTS at the level of area postrema (AP). CCK and ENK were visualized by incubating sections in a mouse monoclonal antibody against CCK-8 sulfate plus a polyclonal rabbit antiserum against ENK followed by anti-rabbit (goat)-texas red and anti-mouse (goat) FITC. Mis-matching the secondary antibodies resulted in abolition of staining.

At the caudal extent of AP, Enk-ergic soma were present in the NTS with processes extending dorsally towards AP and ventromedially to the Dorsal Motor Nucleus (DMN). At this anatomic level, only scattered CCK-containing soma were observed abutting AP. CCK fibers were also localized to the DMN, and several fibers appear to contain both peptides. CCK and ENK fibers were numerous in the transitional area between the medial NTS and the most rostral extent of nucleus gracilus. The number of CCK-soma increased dramatically at the rostal extent of AP and were juxtapositioned between ENK-soma. Interestingly, fibers labeled for one peptide often appeared to encircle soma containing the other peptide. Supported by NS-12311 (BKH), RSDA MH-00595 (PLF).

481.7

VAGOTOMY BLOCKS INSULIN SPIKE THAT PRECEDES MEAL INITIATION. L.A. Campfield, F.J. Smith, D.W. Driscoll* and N. Spirt*. Neurobiology and Obesity Research, Hoffmann-La Roche, Nutley, NJ 07110.

We have previously reported a small, transient rise in plasma insulin (I) prior to the transient decline in blood glucose (TDBG) that precedes meal initiation (MI) in free-feeding rats. We have also shown that following vagotomy, TDBG are not always followed by MI. In order to further characterize the role of I in MI, plasma I and meal pattern were monitored in chronically cannulated, weight matched, male Wistar rats with sham (S) or total subdiaphragmatic vagotomy (V). Blood was continuously withdrawn (25 µl/min for up to 100 min) from lightly heparinized awake rats and pooled over 4 min intervals. In experiments without MI, no significant changes in plasma I were observed over the sampling period in both S and V rats. In experiments (n=6) with MI in S rats, I rose to a peak (60%) then declined to a minimum level at 26 and 8 min prior to MI respectively. In contrast, in V rats I did not rise but instead gradually fell to a plateau prior to MI (n=6). These data demonstrate that a vagally dependent I spike is not necessary for MI. Since we observed an increased frequency of small TDBG that were just at or below threshold for normal MI in V rats, these data suggest that V may have two effects that result in less than faithful coupling of MI and BG: 1) denervation of peripheral glucoreceptors and 2) absence of I spike that may enhance the magnitude of TDBG.

481.8

REVERSAL OF THE ANORECTIC EFFECTS OF CHLOROCITRIC ACID BY SUBDIAPHRAGMATIC VAGOTOMY. W.B. Laughton, D. Driscoll*, and J. Rossi*. Neurobiology and Obesity, Hoffmann-La Roche Inc., Nutley, NJ 07110.

It has been reported that the anorectic effect of the putative satiety agent cholecystokinin (CCK-8) is critically dependent upon the integrity of abdominal vagal sensory fibers. This has been interpreted as evidence for a peripheral site of action of CCK-8.

The present experiment evaluated the role of the vagus nerve in mediating the anorectic effect of chlorocitric acid, another inhibitor of food intake which has been postulated to act at a peripheral site.

Male Sprague-Dawley rats (230-250 g) were subjected to either total subdiaphragmatic vagotomy or sham surgery. Following recovery, animals were food deprived for 17 h (overnight). At 9 a.m., animals were administered either chlorocitric acid (100 mg/kg in dH20 by gavage), or citric acid (same dose) as a control. Thirty-minute food intake was suppressed by 40% in the sham-operated rats ($P < 0.05$); this effect was totally abolished in the vagotomized animals (4% suppression; NS). The effect of 8 µg/kg (IP) CCK-8 (46% suppression) was also totally reversed by vagotomy (1.6% suppression).

These data support the idea that the appetite suppressant effect of chlorocitric acid is a phenomenon of peripheral origin which is relayed to the CNS via the subdiaphragmatic vagus nerve.

481.9

VAGOTOMY ATTENUATES SUPPRESSION OF SHAM FEEDING INDUCED BY INTESTINAL NUTRIENTS. Daniel P. Yox, H. Stokesberry* and R.C. Ritter. Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, Wa. 99164.

We examined the participation of the subdiaphragmatic vagus nerve in the suppression of sham feeding induced by intraintestinal (IIN) nutrient infusions or by intraperitoneal (IP) injection of CCK-8. Infusions or injections were made while rats were allowed to feed with an open gastric fistula. Results are as follows:

	% Suppression of Sham Feeding (±SE)	
IIN or IP	Sham Vagotomy	Vagotomy
CCK-8 (2 ug/kg)	51.2±7.2	10.0±3.5
maltose (0.13 kcal/ml)	27.9±9.4	-4.7±9.0
oleate "	63.2±9.6	1.9±10.3
L-phe "	55.2±4.6	21.7±8.0.

Our results indicate that the subdiaphragmatic vagus nerve is the predominant neural substrate mediating the suppression of sham feeding induced by IP CCK-8 or by IIN maltose or oleic acid. Nonvagal as well as vagal subtrates are involved in suppression of food intake by L-phenylalanine. These results extend our previous findings using the neurotoxin capsaicin and suggest that small unmyelinated vagal sensory neurons mediate the suppression of feeding by intestinal chemical stimuli.

481.10

LOW CAPSAICIN DOSES THAT SELECTIVELY ATTENUATE CHOLECYSTOKININ-SATIETY CAUSE SELECTIVE DEGENERATION OF VAGAL SENSORY TERMINALS. E.H. South, R.C. Ritter, T. Dinh* and S. Ritter. WOI Regional Program in Vet. Med., Univ. of Idaho, Moscow ID 83843 and Dept. of VCAPP, Washington State Univ., Pullman, WA 99164

High doses of capsaicin cause degeneration of primary sensory teminals in the nucleus of the solitary tract (NST) and the spinal nucleus of the trigeminal (SP5). The NST degeneration is associated with attenuation of vagally mediated cholecystokinin (CCK)-satiety. SP5 degeneration is associated with loss of trigeminally mediated corneal chemosensation. To attain more selective behavioral effects of capsaicin on food intake and to attain a better appreciation of the anatomical distribution of CCK-responsive vagal sensory neurons, we compared effects of low capsaicin doses (LCD) (20-25mg/kg) and high capsaicin doses (HCD) (225mg/kg) on the corneal chemosensory response and the suppression of food intake by CCK. Both LCD and HCD attenuated CCK-induced suppression of food intake. LCD but not HCD-treated rats retained corneal chemosensitivity. Rats sacrificed immediately after LCD exhibited a more circumscribed degeneration pattern in the NST than that observed in HCD rats and little or no degeneration in SP5. HCD rats exhibited extensive degeneration in SP5. These data indicate that low intraperitoneal capsaicin doses are relatively selective for destruction of abdominal vagal sensory neurons such as those which mediate suppression of feeding by CCK.

481.11

INTRAGASTRIC (IG) ASPARTAME DOES NOT INFLUENCE SHORT-TERM FOOD INTAKE (FI) OR SELECTION WHEN ADMINISTERED WITH A CARBOHYDRATE (CHO) LOAD. R.J. Bialik, E.T.S. Li and G.H. Anderson. Dept. of Nutritional Sciences, University of Toronto, Toronto, Ontario M5S 1A8.

High doses of aspartame (the methyl ester of phenylalanine and aspartate) have been reported to alter post-absorptive events associated with ingestion of CHO. The present study investigated whether aspartame would alter the normal feeding responses (i.e. FI suppression, diet selection) observed following a CHO load. Rats were adapted to a 12 h (1800-0600) feeding schedule, with a choice of low (5%) and high (55%) protein diets available only during the dark. On different days, rats received either CHO or CHO plus aspartame by gavage. The CHO load (pure cornstarch, 4 ml/kg of a 37.5 g/dl solution) reduced total FI (by 36%) and consumption from the low protein diet (by 43%) in the first hour, without affecting 2 or 12 hr FI. Addition of aspartame (0, 50, 200 or 500 mg/kg) to the CHO load failed to alter FI compared to CHO alone. We conclude that the reduction in total FI and the selective suppression of a low protein diet following a CHO load are unaltered by large doses of aspartame.
(Supported by NSERC of Canada)

481.12

A GLUCOSE INDEPENDENT PROBE UNCOUPLES MEAL INITIATION FROM BLOOD GLUCOSE DYNAMICS. F.J. Smith, D. Driscoll* and L.A. Campfield. Neurobiology and Obesity Res., Hoffmann-La Roche, Nutley, NJ 07110.

A role for peripheral and central glucose receptive neurons in the control of meal initiation (MI) has been proposed. We have previously shown that exogenous glucose infused during a transient decline in blood glucose (BG) delays MI. Glucose and glucose analogs such as 2-buten-4-olide(2B40) have been reported to decrease the firing rate of both hepatic vagal afferents and glucose sensitive neurons. The ability of 2B40 to block MI was assessed in free-feeding female Wistar rats chronically implanted with cardiac and femoral cannulas. BG and meal pattern were continuously recorded (up to 150 min) following a 2 min IV infusion of 10, 20, 40 or 80 µM of 2B40. During intermeal intervals, changes in BG were not observed following administration of 10, 20 and 40 µM (<5%) but increased by 30% following 80 µM; feeding was not observed in any of these 24 trials. When 2B40 was superimposed on transient declines in BG, MI was reliably blocked following 20 and 40 µM and only partially (33%) blocked following 10 µM. In contrast to glucose, application of 2B40 (10-40 µM) did not alter the shape of the decline in BG, suggesting that uncoupling of MI from BG occurred at the level of detection by glucose sensitive neurons. We conclude that 2B40 uncouples MI from BG at low doses by blocking the detection of declines in BG and at higher concentrations by also altering BG dynamics.

482.1

EVIDENCE THAT THE CELLULAR SOURCE FOR RETINAL REGENERATION IN GOLDFISH IS WITHIN THE DIFFERENTIATED RETINA. P.A. Raymond. Dept. Anat. & Cell Biol. and Neurosci. Prog., Univ. Michigan, Ann Arbor, MI 48109.

The neural retina in fish and amphibians can regenerate following surgical or neurotoxic destruction. We have proposed that rod precursors are the source of new cells for retinal regeneration in goldfish. Rod precursors are proliferating cells within the differentiated retina that normally produce only rods. Other proliferating pools that are potentially involved in regeneration include the circumferential retinal germinal zone (GZ) and the retinal pigmented epithelium (RPE). To determine whether cells from the GZ disperse and migrate centrally to colonize degenerated regions, a cocktail of ^{3}H-thymidine (which labels mitotic cells in the GZ) and ouabain (which destroys differentiated retinal neurons) was injected into the right eye (RE), and ^{3}H-thymidine alone was injected into the left (LE). One month later both eyes were prepared for autoradiography. In the LE, a wedge of heavily labeled cells separated the original retina from the peripheral annulus of new retina added at the GZ by normal growth. In the RE, a diffuse wedge of weakly labeled cells was at the boundary between central, degenerated (now regenerating) retina and a peripheral annulus of new retina. The weak label reflected increased proliferative activity in the GZ of the RE, but the extra cells contributed to peripheral growth, not central regeneration: the annulus of new retina added by the GZ was about 220 µm wider in the RE. The degenerated (central) retina had collapsed, however, so overall retinal length was about 400 µm less in the RE. In amphibians it is thought that central retina regenerates from RPE following retinal degeneration induced by devascularization. We have used ouabain to destroy retinal neurons, which might account for the difference in results. I report here that in devascularized goldfish eyes, there is no evidence for RPE involvement in regeneration; instead the process is similar to that described previously for ouabain-treated retinas. These results provide further evidence that neither GZ nor RPE is responsible for retinal regeneration in fish.

482.2

RABBBIT RETINAL GANGLION CELLS SURVIVE OPTIC NERVE TRANSECTION. C. Rosario*[1], K. Fry[2], and R. Madison[1]. (SPON: B. Seckel). Departments of Neuropathology and Neuroscience, Harvard Medical School and Children's Hospital, Boston, MA.[1], and Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX.[2]

Several recent reports have suggested that under certain conditions rabbit retinal ganglion cells will survive optic nerve transection (Lavie et al., Brain Research, 419, 166-172, 1987). For the past several years this laboratory has been investigating the applicability of various "nerve guide tubes" to effect nerve regeneration in the peripheral nervous system (see Abstracts by Arichibald and Madison, and Krarup et al. this volume). The current study assess the usefullness of such nerve guide tubes to effect repair of the central nervous system, the rabbit optic nerve.

Six adult male New Zealand rabbits received transection of the left optic nerve intra-orbitally, immediately distal to the papilla. A collagen based nerve guide tube (1 cm) was sutured onto the short proximal nerve stump, and the tube was filled with Type I collagen (Vitrogen) containing 1000 units/ml acidic fibroblast growth factor with heparin (gift of Dr. P. D'Amore). Eight to 10 weeks following surgery, animals were processed for either frozen section immunocytochemistry with the AB5 antibody which is specific for retinal ganglion cells (Fry et al., Brain Research, 338, 360-365, 1985) or plastic embedding. Histological results indicate that under these conditions, although many ganglion cells have apparently died, there is a distinct population of AB5 positive cells which continue to remain in the retina. Supported by NS22404 to RM.

482.3

A NOVEL TREATMENT CAUSES REGENERATION OF AXONS AMONG ASTROCYTIC PROCESSES WITHIN THE ADULT RABBIT OPTIC NERVE. M. Schwartz, M. Belkin*, A. Solomon*, V. Lavie, S. Ben-Bassat*, M. Murray[1], M. Rosner*, A. Harel* and S. Rumlet*, Weizmann Institute of Science, Rehovot, Israel and [1]Medical College of Pennsylvania, Philadelphia, PA

Degenerating CNS tissue is apparently inhospitable to growing axons. Our results suggest, however, that it can be amenable to activation to become supportive for regeneration. Regenerating fish optic nerves can provide activators, which caused a marked increase in laminin immunoreactive sites in treated injured rabbit optic nerves. However, it is not sufficient to bring regeneration to completion. To get a better growth, we have combined the application of the activators with low energy He-Ne laser irradiation, which had a delaying effect on injury-induced degeneration. Such treatment has resulted in the appearance of abundant newly growing unmyelinated axons, which traversed the site of injury and extended into the distal nerve stump. These axons were seen adjacent to astrocytes. About 4000 axons were counted in the treated nerves, six weeks after injury. These axons appeared in a compartment (4.8% of the total cross-section area), consisting of 47.9% of unmyelinated axons and 41.9% of myelinated axons. In contrast, in non-treated nerves six weeks after injury no axons could be observed, though the lesions were comparable. Identification of these cells (now supporting growth) using specific markers for glial cells at various stages of differentiation is currently under way.

482.4

PATHWAY SELECTION BY REGENERATING MAUTHNER AXONS OF THE ADULT GOLDFISH. S.J. Zottoli, M.A. Agostini*, P.D. Danielson*, E.J. Lee*, T.L. Laidley*, E.A. Markstein* and T.L. Scalise*. Dept. of Biology, Williams College, Williamstown, MA 01267.

Injury to the spinal cord of adult goldfish results in the loss of function caudal to the wound. Although functional recovery of swimming is known to occur, the sound-evoked C-bend initiated by the Mauthner cell (M-cell) does not return for at least 6 months. In an attempt to determine the morphological basis for the lack of this functional recovery we have severed the M-axon by spinal cord crushes or cuts and studied the pathways taken by its sprouts. M-axons were filled with Lucifer yellow and studied in brain wholemounts 30 and 60 days postoperatively (22°C). Eighty-five percent of the filled M-axons regenerated and 52% of those axons sent sprouts caudally as well as rostrally. The longest sprouts extended 3.0mm rostrally and 3.6mm caudally from the retraction bulb. Some of the caudal projections extended past the wound site for up to 3.0mm and the majority of these sprouts extended ventral and lateral to the previous location of the M-axon in the spinal cord. Since none of these fish displayed sound evoked C-bends, we hypothesize that regenerating M-axons follow pathways in the spinal cord that are inappropriate for functional regeneration. Supported by NSF grant BNS 86-06607 and CSI 86-50488.

482.5

Functional regeneration demonstrated in the adult spinal cord of the lamprey. A.H. Cohen, M.T. Baker*, T.A. Dobrov*.Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

It is known that there can be functional regeneration in larval lampreys (Cohen et al., PNAS.83:2763-2766,1986). We now show that adults with spinal lesions share this capacity for regeneration, but incompletely.

Partial lesions of either lateral or medial tracts were made in young feeding adult lampreys (*Petromyzon marinus* and *Ichthyomyzon unicuspis*). After 8-10 months, the animals were tested for functional regeneration. The spinal cord was dissected out with the notochord and superfused with curare (15mg/l) and D-glutamate (0.25-0.50 mM) to activate fictive swimming. The activity of two motor nerves, one rostral and one caudal to the lesioned segment, were monitored. When stable and coordinated, the bursting was recorded. The tracts which had formerly been spared were cut in the lesioned segment and bursting again observed. 200 consecutive bursts of the most stable coordinated bursting were recorded and analyzed and the phase lags between the two segments were measured. Plots of the phase lags over time and phase histograms were made. The non-randomness of the histograms was assessed by a Chi-square test with 19 degrees of freedom (distributing the phase lags over 20 equal bins). After the experiment was completed the cords were examined histologically to assure that the fibers remaining in the lesioned segment all had the typical regenerated appearance. Of the medially lesioned animals 3 of 5 showed non-random distributions of phase lags during fictive swimming (Chi-square 23.7-116.8; $p<<0.001=38.6$). 3 of 5 laterally lesioned also showed non-random phase delays (Chi-square 19.1-296.7). Thus, many questions remain, but it can be said with certainty that adult lamprey spinal cords retain some capacity for functional restoration of fibers which can sustain intersegmental coordination during drug induced fictive swimming even in the absence of sensory, mechanical and descending factors. Supported by NIH grant No. NS16803 and AFOSR contract No.F49620-87-C-0013.

482.6

REGROWTH OF AXONS INTO AN EXTENDED CELLULAR MATRIX FOLLOWING INJURY OF THE DORSAL COLUMN OF THE ADULT RAT SPINAL CORD BY A METHOD OF CONTROLLED FREEZING. N.R. West and G.H. Collins. Departments of Pathology and Anatomy, SUNY Health Science Center, Syracuse, New York.

With a proper matrix it is known that axons of mature mammalian central nervous system regenerate. It has been more difficult to demonstrate that such a matrix can develop from supporting elements of central nervous tissue. We were able to demonstrate regrowth of axons into a matrix 2.5-3.0 mm in length following cryogenic injury to the dorsal column of mature rat spinal cord (Collins, West, et. al. J Neuropathol Exp Neurol 45:742, 1986). Recently, we demonstrated the feasibility of producing, by this method, a lesion 1.0 cm in length within which a similar matrix develops (West and Collins, Neuroscience Abstracts, 1987). The purpose of this report is to demonstrate that axonal regrowth will occur through a matrix which is 5-10 mm in length. These results are based upon the study of 5 animals subjected to cryogenic injury of the dorsal column at T7-T9, using cryodes 4.0-7.0 mm in length. Fifteen days following injury animals have lesions 5-10 mm in length. Macrophages are sequestered into identifiable areas, around which a cellular matrix containing myelinated and unmyelinated axons is seen. The axons which diminish in the proximo-distal (caudal-rostral) direction, represent a regrowth distance of 5.0-10.0 mm.

482.7

INDUCED REGENERATION OF CUT DORSAL ROOT FIBERS INTO ADULT RAT SPINAL CORD. J.D. Siegal* , M. Kliot, G.M. Smith*, S. Tyrrell*, J. Silver (SPON: E. Housepian). Neuroscience Center, CWRU Medical School, Cleve., OH 44106.

Glial scar formation and inhibition by white matter are thought to impede sensory axons from regenerating across the dorsal root entry zone (DREZ) into the adult rat spinal cord. In order to assess the relative roles of these barriers, we unilaterally transected lumbar dorsal roots (L4-L6) and implanted the distal portion of L4 medially into the white matter of the dorsal columns or laterally just superficial to the gray matter of the dorsal horn. After 3 weeks, anterograde labelling with HRP demonstrated that many axons entered the spinal cord from laterally-implanted roots. A significant population of these axons entered the gray matter and formed terminal fields with synaptic boutons. Other axons extended into the white matter of the dorsal columns for variable distances. The placement of a specially designed Millipore filter, coated with embryonic astrocytes, just medial to the implanted root increased regeneration into the gray matter. Successful regeneration of axons was associated with a localized and limited inflammatory response near the sites of ingrowth. We, therefore, believe that axonal regeneration is enhanced by the lateral placement of a transected sensory root, the use of an astrocyte-coated polymer, and the presence of a limited inflammatory response. NEI-EY05952.

482.8

ENHANCED DORSAL ROOT AXONAL REGENERATION IN ADULT RAT SPINAL CORD FOLLOWING ENTRY ZONE IRRADIATION. F.J. Liuzzi, Dept. of Anatomy and Cell Biology, Eastern Virginia Med. Sch., Norfolk, VA 23501.

After dorsal root crush the majority of regenerating axons are stopped at the root-cord interface where they encounter the CNS environment of the cord. EM of this region reveals tightly interwoven astrocytic processes and numerous astrocytic somata. Although many of the regenerating axons terminate among the astrocytic processes to form stable axo-glial endings (Liuzzi and Lasek, Science 237:642, 1987), HRP anterograde injury-filling reveals that a small number of these regenerating axons breach the entry zone to gain access to the spinal gray matter (Liuzzi and Lasek, Soc. Neurosci. Abst. 13:395, 1987).

I have begun to study the use of post-crush irradiation of the dorsal root entry zone as a way of modifying the glia of the region with the intention of enhancing the regeneration of axons into the cord. In 3 of 4 animals examined to date, there has been an apparent enhancement of axonal growth into the cord as compared to that in non-irradiated animals. HRP labelling of regenerating dorsal root axons revealed a variable reinnervation of the spinal gray matter. EM of labelled axons in one animal revealed Schwann cell myelination in the white matter and identifable HRP-labelled synaptic terminals in the gray matter. This work is supported by grants from the SCRF and NINCDS.

482.9

DORSAL ROOT IMPLANTS IN PREDEGENERATED RAT SPINAL CORD. A. Bignami, H. Mansour* B. Labkovsky* and D. Dahl Spon: J. Bossom. Harvard Medical School and VA Medical Center, Boston, MA 02132.

Glial hyaluronate binding protein (GHAP) is a brain specific protein localized in white matter astrocytes and showing structural similarity (up to 89%) with the hyaluronate binding region of cartilage proteins (see Perides et al., this meeting). GHAP disappears from reactive astrocytes in long-standing Wallerian degeneration. Extensive dorsal rhizotomy was performed in rats at the thoracolumbar level. One month after operation, 2 dorsal roots (not previously transected) were implanted into the degenerated dorsal columns. The rats were examined at varying intervals after the second operation. In 3 rats, the implanted roots were found within the degenerated dorsal columns. Double staining experiments showed that regenerated axons crossed the boundary between laminin-positive PNS and laminin-negative CNS. Furthermore, the periphery of the implant was strongly positive with both laminin and GFAP antibodies, thus indicating invasion by reactive astrocytes. Regenerated axons followed an irregular course in the severely gliosed tissue surrounding the implant. No growth was observed in the degenerated dorsal columns above the zone of implantation. The findings suggest that normal astrocytes rather than reactive astrocytes form a barrier to axonal growth. Supported by NS 13034 and by the VA.

482.10

EVIDENCE FOR REINNERVATION OF SKELETAL MUSCLE BY MOTONEURONS VIA VENTRAL ROOT IMPLANTS INTO THE SPINAL CORD OF THE CAT. T. Carlstedt , S. Cullheim*, H. Lindå*, M. Risling* and B. Ulfhake* Dept of Handsurgery, Dept of Anatomy, Karolinska Institutet, Box 60400, S-10401 Stockholm, Sweden.

We have previously shown that cat lumbar motoneurons may reinnervate ventral roots after division of their axons in the spinal cord (Risling et al., Brain Res. 280:15, 1983). This unexpected regenerative capacity of the motoneuron has been further explored in cats after implantation of avulsed ventral roots into the spinal cord.

The left ventral roots L6-S1 were avulsed from the surface of the cord. The L6 root or rostral L7 rootlets were immediately inserted into the ventrolateral part of the L7 segment. After 11-12 months, spinal neurons responding antidromically to stimulation of implanted roots were injected intracellularly with horseradish peroxidase and were studied light and electron microscopically.

Morphological and physiological analysis of the studied neurons revealed that alpha and probably also gamma motoneurons were able to reinnervate ventral root implants. Such neurons could be excited or inhibited by segmental reflex activity and their axons could conduct nerve impulses. The character of muscle twitch responses, elicited by electrical stimulation of implanted roots, strongly indicated that denervated muscles were reinnervated by new motor axons via the transplant.

482.11

REGENERATION OF CENTRAL AXONS INTO PERIPHERAL NERVE GRAFTS Keith Carson, J.K.Terzis,D.Spurrier*,T. Liberson*, C.Peffley* Microsurgical Research Center,E.V.M.S, Norfolk, VA 23507

At present, the possibility of surgical intervention to restore lower limb function in the paraplegic patient has been limited. The major limitation to restoring motor function has been the absence of a suitable motor neuron pool that can be tapped in order to replace motor neurons located caudal to the site of spinal injury. We wish to address the question of whether central neurons, other than those of the ventral horn, can contribute regenerating axons to denervated peripheral musculature. To study this problem, we used peripheral nerve grafts to connect the spinal cord to a denervated distal muscle (the gastrocnemius) in the rat. The left sciatic nerve is dissected out as a free nerve graft and the distal end inserted into the corticospinal tract through an incision in the dorsal column and the proximal end is coapted with the contralateral sciatic nerve at the sciatic notch. Following a six month survival period, the origins of the CNS neurons sending axons into the peripheral nerve graft are determined by using HRP as a retrograde tracer. The purposes of this experiment include: 1) to confirm whether central neurons can regenerate new axons into peripheral nerve grafts inserted into the spinal cord; 2) to confirm that at least some of these axons can re-establish functional contacts with peripheral targets; 3) to determine the central origins of fibers that invade the PNS grafts; and 4) to examine the morphological, physiological and behavioral extent to which motor function of reinnervated muscles is restored. Preliminary light microscopy through the distal region of the graft shows hundreds of myelinated axons. Currently HRP transport studies are in progress to trace the cells origin of the axons.

482.12

CELLULAR ORIGIN OF THE REGENERATIVE PLACODE DURING LATERAL LINE REGENERATION IN THE AXOLOTL. J. E. Jones* and J. T. Corwin. (SPON: H. Gillary) Békésy Lab. of Neurobiology, Univ. of Hawaii, Honolulu, HI 96822

Neuromast organs in lateral line systems contain sensory hair cells and supporting cells that are similar to their counterparts in the ear. Partial tail amputation in the axolotl (Ambystoma mexicanum) results in regeneration of the lost portion and replacement of the neuromasts removed. Previous work showed that an undifferentiated cellular mass, the regenerative placode, originates from the distalmost neuromast remaining after amputation and gives rise to the new neuromasts. We identified the type of cell that initially seeds the placode, and determined how the placode advances during regeneration. DIC microscopy, combined with time-lapse videorecording, documented that cells on the posterior margin of the deepest layer of supporting cells in the last neuromast remaining after a tail cut became mitotically active at about the time the placode normally appears. Cell divisions were frequent in the placode itself, once seeded by these basal layer supporting cells. The production of new cells must account for some part of the placode's extension, but its cells also became motile, as evidenced by membrane ruffling. Ruffling is usually a result of lamellipodia-mediated cell migration. During wound healing in mammals, ruffling is seen at the leading edge of the advancing epithelial sheet. The process differed here in that all cells within a given area were involved. Macrophages were found to be present and active at the regeneration site. In other systems these cells have been shown to secrete specific mitogens, suggesting the possibility of such a role here. (Supported by grants from NINCDS to J. T. Corwin.)

483.1

REGIONAL APPEARANCE OF MYELIN CONSTITUENTS IN THE DEVELOPING RAT SPINAL CORD. M.E. Schwab and T. Savio. Brain Research Institute, Univ. of Zurich, August-Forel-Str. 1, CH-8029 Zurich, Switzerland.

The regulation of oligodendrocyte differentiation and myelin formation is poorly understood at present. On the other hand, oligodendrocytes contain specific membrane proteins able to inhibit nerve fiber growth (Caroni and Schwab, '88). Studying the appearance of myelin specific glycolipids (GalC) and of MBP in the rat cervical spinal cord we found that the time of expression of these components is highly specific for a given structure. It starts in the ventral funiculus (P1) and dorsal ascending tracts (P2-P4). Pyramidal tract (P11) and gray matter regions (patchy appearance!) myelinate late. - Frozen sections of spinal cords were used as substrates for cultured neuroblastoma cells and neurons. On adult and P13 tissue white matter areas were strongly inhibitory for cell adhesion and neurite growth. In contrast, all regions of P0 spinal cord were good substrates. When oligodendrocytes were suppressed P13 spinal cords did not exhibit inhibitory substrate properties. These results suggest that local signals in fiber tracts crucially influence oligodendrocyte differentiation, and that the expression of inhibitory substrates could locally influence nerve fiber growth.

483.2

CHANGES IN PROTEASE NEXIN I (PNI) DURING C_2 MUSCLE CELL DEVELOPMENT. B.W. Festoff, C. Maben*, and J.S. Rao*. Neurobiol. Res. Lab (151), V.A. Med. Ctr., Kansas City, MO 64128.

PNI inhibits the activity of serine proteases and also mediates their binding, internalization and degradation by cells. PNI is identical to glial derived nexin (GDN), a neurite outgrowth promotor found in rat and human glial cells. Our results show the increased production of PNI in post-fusional myotubes compared with myoblasts. The increased production of PNI was characterized quantitatively by ELISA and immunoprecipitation. ELISA shows that PNI production increases at day 4 after fusion. ^{35}S-labeled myoblast and myotube media and cell layer extracts were passed through a heparin-Sepharose column, the eluate was precipitated with anti-PNI and characterized by SDS-PAGE. In peak 1 (0.2M NaCl), 43 Kd protein was increased at day 4, while in peak 2 (0.45M NaCl), 48 Kd was increased at day 4. These results show the increased production of two different molecular forms of the serpin, PNI, during myogenesis in vitro.

Supported by the MDA, ALS Assoc., NSF, & the Medical Research Service of the VA.

483.3

ELECTRON-HISTOCHEMICAL OBSERVATION OF 5'-NUCLEOTIDASE IN PRIMARY CHICK EMBRYONIC NEURAL CULTURES H.C.LUDWIG,P.E.SPOERRI*and E.MARKAKIS.Department of Neurosurgery,Georg-August-University,Robert-Koch-Str.40,D-3400 Goettingen,Federal Republic Of Germany

5'-nucleotidase has shown in several investigations to be an ubiquitous enzyme present in neuro nal and nonneuronal particularly glial plasma membranes(Kreutzberg et al.,Brain Res.158,1978;247). The ectoenzyme may play an important role in neural-glial interactions in which glial cells are providing adenosine to the neurons and therefore facilitate neuronal growth,development and differentiation.Presently,the expression of 5'-nucleotidase was examined in reaggregating neurons and glial cells,isolated from 8 day old embryonic chick brain,spinal cord and retina grown in monolayer culture systems.By modification of known cytochemical methods for EM demonstration of 5'-nucleotidase we were able to localize the enzyme on the plasma membranes of glial flat cells.Special interest was directed towards the heterogeneity of the membranes of retinal Mueller cells grown in histotypically oriented rosettes.In these rosettes the degree of heterogenious expression of 5'-nucleotidase coincides with developing neurons and retinal receptors.

483.4

OMEGA-3 FATTY ACIDS IN THE DEVELOPING PHOTORECEPTOR CELL. N. G. Bazan, F. Cai* and B. L. Scott*. LSU Eye Center, New Orleans, LA 70112

The omega-3 essential fatty acid family comprises a major acyl group of retinal photoreceptor membranes and synaptic membranes of brain and retina. Docosahexaenoic acid (22:6w3) represents nearly 50% of the fatty acyl groups in the disk membrane of photoreceptors although its function is not well understood. We are studying the omega-3 fatty acid family in the developing mouse pup and find that the major omega-3 fatty acid supplied to pups in mother's milk is linolenic acid (18:3w3). In addition we have studied the accumulation of 22:6 in developing photoreceptor cells and brain of mice at different postnatal ages and have examined the ability of these animals to synthesize 22:6 from 18:3.

We find that the content of 22:6 in photoreceptor cells increased 3- to 4-fold in the various phospholipid classes as the cells synthesize the adult complement of rod outer segment disk membranes. Furthermore, studies of the synthesis of $[^{14}C]$22:6 from intraperitoneally-injected $[^{14}C]$18:3 indicate that the liver is the major site of this conversion in the developing pups, and that omega-3 fatty acid is presented to the growing photoreceptor cells and to brain primarily in the 22:6 form. A similar model is postulated also to occur during the differentiation of synaptic membranes. Supported by EY04428 and the Edward G. Schlieder Educational Foundation.

483.5

EXPRESSION OF A 35-kDa SUBSTRATE FOR THE EGF RECEPTOR KINASE IN A MIDLINE RAPHE OF THE FLOOR PLATE PRIOR TO THE ARRIVAL OF DECUSSATING FIBERS IN RAT EMBRYOS. James A. McKanna* (SPON: J.A. Pulliam), Dept. of Cell Biology, Vanderbilt Univ., Nashville, TN 37232

A 35-kDa protein (p35), purified by Fava and Cohen (JBC 259:2636) as a preferential substrate for the tyrosine kinase activity of the EGF receptor, is developmentally regulated in embryos and is presumed to play a role in morphogenesis. In rat embryos of 11 days gestation (E11), p35 is expressed in a ventral midline raphe of primitive glial ependymal cells that run from the lumen of the neural tube to the pial surface. The p35 staining is first apparent in the rostral hindbrain, but by day E14 the raphe runs the length of the floor plate from the caudal edge of the midbrain to the tip of the spinal cord. Thus, p35 is expressed in glial cells at the future ventral commissure prior to the arrival of decussating axons, and it may serve gradient, guide or gate functions. The p35 immunoreactivity disappears from the CNS by postnatal day 3; however, because silver stains show that the raphe cells persist through adulthood, we prefer to avoid the term "radial glia" which refers to cells that purportedly differentiate. In addition to serving as a tyrosine kinase substrate in the mediation of growth factor effects, p35 binds calcium and phospholipids, indicating that it may influence the cytoskeleton. Supported by CA43720.

483.6

BASIC FIBROBLAST GROWTH FACTOR STIMULATES SURVIVAL OF NON NEURONAL CELLS DEVELOPING FROM TRUNK NEURAL CREST. C. Kalcheim Dept. of Anat.& Embryol. Hebrew Univ. Hadassah Med.School-Jerusalem 91010-Israel.

Our study characterizes the early influence of the CNS on survival of neural crest (NC) cells. We find that basic fibroblast growth factor (ß FGF), synthesized in the CNS, stimulates survival of HNK-1 immunoreactive NC cells separated from the neural tube by a silastic membrane impregnated in 100 ng/ml ß FGF. Rescued cells were then observed for over 30 hr. after grafting in 12 out of 16 chick embryos. In contrast, no surviving cells could be found in any of 10 control embryos. ß FGF was also tested on trunk NC cells cultured with somite cells. FGF promoted a dose dependent increase in the number of HNK-1 positive non neuronal cells in 1-4 day old cultures (1.8 to 8.2 fold over controls using FGF at 10 pg/ml to 1ng/ml). Similar increase in the number of non neuronal cells was attained when FGF was added to pure NC cultures. FGF had no mitogenic effect on the NC-derived non neuronal cells but found to increase the incorporation of (3H) Thymidine into acid insoluble material in somite cultures devoid of NC by 3.3 - 8.4 fold over control. Our results demonstrate a direct effect of FGF on survival and/or differentiation of the NC-derived non neuronal cell population. Sponsored by the MDA and The Israel Acad. of Sciences.

483.7

DIFFERENTIAL EXPRESSION OF THE CHICKEN NERVE GROWTH FACTOR RECEPTOR GENE DURING DEVELOPMENT. E Escandon* and M Chao*.(SPON:L. Schleifer). Dept. Cell Biol. and Anatomy, Cornell University Med. College, New York, NY 10021.

A genomic chicken library was screened with human nerve growth factor (NGF) receptor cDNA probes at moderately high stringency. Four positive bacteriophage clones were isolated and characterized by restriction mapping and Southern blot analysis. A 5.5 kb Hind III fragment was isolated by virtue of homology with the 5' end of the human cDNA sequence. The region of homolgy was further localized, analyzed by DNA sequencing and found to correspond to the extracellular domain of the NGF receptor. Northern blot analysis of poly (A)+ RNA from embryonic chicken brain detected a unique and specific mRNA of 3.9 Kb in length, similar to the size seen in human melanoma cells, rat PC12 and Schwann cells. The same mRNA could also be detected in chick spinal cord and dorsal root ganglia. S1 nuclease protection analysis indicated that NGF receptor mRNA is expressed in the various brain vesicles and their derivatives, substantially decreasing at later embryonic stages. The differential appearance of receptor mRNA and the presence of NGF in selective brain regions suggests a role for NGF and its receptor during chicken brain development.

483.8

GABA AND $GABA_A$/BENZODIAZEPINE RECEPTOR-LIKE IMMUNOREACTIVITY IN THE DEVELOPING RAT CEREBELLUM. D.L. Meinecke, and P. Rakic. Section of Neuroanatomy, Yale School of Medicine, New Haven, CT 06510

To explore the possibility that the development of local neuronal circuits may be related to the expression of neurotransmitters and their receptors, we have studied the ontogeny of GABA and $GABA_A$ receptors in cells which form GABAergic inhibitory connections in the rat cerebellum. Cerebella from rat pups ages 0 days to 21 days post-natal were immunostained with GABA antisera (Immunonuclear) and E9 monoclonal antibodies directed against the $GABA_A$/benzodiazepine receptor complex (Sweetnam et al., Mol. Brain Res. '87). Immunolabeled tissue was examined with light and electron microscopy.

The cerebellum of new born animals contains only pre-natally generated Purkinje and Golgi II neurons. At this early stage, both cell types could be immunolabeled with GABA and E9 antibodies. The other cells of the cerebellar cortex arise post-natally from a germinal external granular layer which is present from about 2 to 21 days. Cells of the external granular layer and descending migrating neurons were not labeled with either GABA or E9 antibodies at any age. However, after arriving in the granular layer, maturing granule cells and their growing dendritic processes were E9 positive. Like granule cells, basket and stellate cells in the molecular layer do not appear to immunoreact with either antibody during their bipolar stage, and express E9 and GABA only after they develop dendrites and axons. These results indicate that cells which participate in GABAergic cerebellar circuits express GABA receptors only after they attain their final position and begin elaborating dendrites involved in synaptogenesis.

Supported by NIH grants NS14841, NS2280.

483.9

TRISOMY 16 MOUSE MODEL OF DOWN'S SYNDROME: 210KDALTON NEUROFILAMENT SUBUNIT REGULATION BY INTERFERON; CENTRAL NERVOUS SYSTEM ELECTRON MICROSCOPIC OBSERVATIONS A. V. Plioplys. Surrey Place Centre and Div. Neurology, Hospital for Sick Children and Univ. Toronto, Ont. M5S 2C2

The trisomy 16 mouse is an excellent model for the human Down's syndrome (DS). It is postulated that sensitivity to interferon may underly defects in neuronal formation in DS (J Neuro Sci 1987, 79:91). When applied to CNS cultures taken from normal fetal mice, interferon increases the immunohistochemical expression of the 210K neurofilament subunit. This effect can be block by the application of oxyphenbutazone which inhibits interferon-mediated metabolic pathways. CNS cultures taken from trisomy 16 fetal mice express greater intensity of the 210K neurofilament subunit immunohistochemical staining than do normals. Application of oxyphenbutazone normalizes trisomy 16 CNS neurofilament expression.***EM observations were made of the cortical plate within the developing telencephalic vesicle at E17. The results included: 1. neuronal microtubular profiles which were more coiled and curved in the trisomic condition than in normals; 2. increased neuronal membrane fragility in trisomy 16; 3. increased nuclear contour irregularity in trisomic neurons; 4. significant decrease in the cross-sectional area of neuronal nuclei in trisomy 16 ($p<0.01$). The observations concerning nuclear morphologic differences in trisomy 16 may be related to the reported differences in nuclear histone expression in Alzheimer's disease.

483.10

A BEHAVIORAL AND ANATOMICAL INNER EAR MUTANT GENERATED BY INSERTIONAL MUTAGENESIS IN TRANSGENIC MICE. E.B. Crenshaw III*, [1,4], A.F. Ryan[2,3], and M.G. Rosenfeld*[4,5]. (SPON: R. Emeson) Depts. of [1]Biology, [2]Surgery/Otolaryngology and [3]Neurosciences, and the [4]Eukaryotic Regulatory Biology Program, UCSD School of Medicine, and [5]Howard Hughes Medical Institute, La Jolla, CA 92093.

Transgenic mice were generated by microinjection of a chimeric gene consisting of the 5' flanking region of the human vasopressin gene fused to the v-src gene. While most of these animals exhibited normal behavior, one of fourteen lines showed circling, hyperactivity and head tossing similar to that seen in some labyrinthine mutants. The mutant integration event in this strain cosegregates with phenotype.

Anatomical examination of the mutant's inner ear showed disorganization of the vestibular membranous labyrinth. Most vestibular epithelia were absent. The saccular macula was reduced in size, but relatively normal in cellular structure. Its otolithic membrane was overgrown with epithelium or characterized by amorphic, enlarged otoconial remnants. The vestibular ganglion appeared to be normal in size, and afferent fibers entered the vestibule in near normal numbers. However, they projected in apparently random directions. The cochlea of the mutant was largely normal in appearance, though loss of spiral ganglion cells in the apical turn varied from slight to a virtually complete loss of neurons in the upper one third of the cochlea.

The ability to clone the gene from this mutant will allow the exact characterization of mutational events, as well as identification of the normal gene involved in those aspects of inner ear development that have been altered in the transgenic mouse.

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483.11

PROLIFERATION OF TREMBLER MOUSE SCHWANN CELLS IN CULTURE AND DURING WALLERIAN DEGENERATION. H.L. Koenig*,N.A. Do Thi* and B. Ferzaz* (SPON : D. Fambrough).Lab. Neurobiologie Development, Univ. Bordeaux I, 33405 TALENCE, France.

Schwann cells (SC),which do not divide in vivo, proliferate in cell cultures when mitogenic signals,such as neurite or myelin breakdown products are present.In Trembler mutation,characterized by hypomyelination and basal lamina excess,the Schwann cells proliferate continually.The increase of SC is regular in the peripheral nerve from 3 day-old mice(+25%) to adult (x6).We were interested to analyse the proliferation capacities of Trembler and control SC when they are deprived of neural influence and after they are re-associated with nervous tissue.In cell cultures, SC from 1 and 4 days animals had a similar nuclei labeling index at each time tested (1 to 9 days in culture).By contrast,when Schwann cells are plated from 15 days old mice, Trembler cells proliferate less than control cells.Similarly, in wallerian degenerating sciatic nerve, the proliferation of Schwann cells is approximatly 5 times higher in control than in Trembler.

When the tibial nerve is sutured to the degenerated distal stump, the SC proliferation is stimulated anew, less in Trembler than in control. In culture, Trembler and control nerve extracts,added respectively to control and Trembler SC stimulate similarly their proliferation at a very high level.

The Trembler mutation affecting primarly the SC provides a good model system to analyse the cellular origin and molecular nature of in vitro and in vivo mitogenic signals.

484.1

DIFFERENT DISTRIBUTION OF SLOW AND FAST K CHANNELS IN RAT MYELINATED NERVE. J.R. Schwarz* and J. Röper* (SPON: Europ. Neurosc. Assoc.). Inst. of Physiol., UKE, D-2000 Hamburg 20, FRG.

Single myelinated rat nerve fibers were dissected and voltage clamped to study the properties of their K channels. K currents were recorded in the intact node of Ranvier and after acute paranodal demyelination with lysolecithin and pronase. Results: 1. The analysis of tail currents recorded in isotonic KCl solution revealed that K channels with slow gating kinetics were located in the nodal membrane whereas those with fast kinetics were located in the paranode. The conductance of the fast K channels increased linearly with the increase in the axonal area which had been demyelinated. 2. The sigmoidal steady state activation curve of the slow K channels had an inflection point at -70 mV, i.e. 50% of these channels were in the open state at the resting potential. At negative potentials they showed an inward rectification which could be reduced by addition of $BaCl_2$. 3. The paranodal fast K channels were activated at more positive potentials and were selectively blocked by 1 mM 4-aminopyridine. Conclusion: Myelination in rat nerve is accompanied by a local segregation of slow and fast K channels.

484.2

TWO LARGE CONDUCTANCE K^+ CHANNELS IN THE SOMATIC AND DENDRITIC REGION OF CEREBELLAR PURKINJE NEURONS. D.L. Gruol, T. Jacquin * and A.J. Yool. Div. Preclin. Neurosci., Res. Inst. Scripps Clinic, La Jolla, CA 92037.

We have previously identified several types of voltage-sensitive K^+ channels in cell-attached recordings from the somatic and dendritic region of cerebellar Purkinje neurons in culture. In the present series of experiments, we have focused on the characteristics of two of these channel types when studied in outside-out and inside-out membrane patches. Using saline solutions mimicking physiological conditions, the single channel conductances were 90 and 75 pS. Both channel types were active at potentials depolarized to the normal resting membrane potential of these neurons (-60 mV) and had extrapolated reversal potentials around -65 mV. Changing the equilibrium potential for Cl^- by ion substitution did not alter the reversal potential for the unitary events; changing the equilibrium potential for K^+ did, demonstrating that the channels were K^+ selective. Both channel types were sensitive to low concentrations of extracellular TEA, channel activity being significantly reduced by 1 mM concentrations. The activity of both channel types was also reduced by 10 mM extracellular Ba^{++}. Thus, the two K^+ channel types are similar in conductance and pharmacological sensitivity. The main characteristic that distinguishes the channel types is voltage sensitivity. The 75 pS channel type is most active at potentials hyperpolarized to 0 mV; the frequency of opening and the duration of the open state increases as the membrane potential is hyperpolarized. In contrast, the 90 pS channel is active at potentials depolarized to 0 mV; the frequency of channel opening and the duration of the open state increases as the membrane potential is depolarized. This difference in voltage-sensitivity suggests that the two channel types play different roles in controlling the excitability of Purkinje neurons. Supported by NS 21777 and training grant AA07456.

484.3

SINGLE POTASSIUM CHANNELS IN CULTURED RAT HYPOTHALAMIC NEURONS. J. G. McLarnon, Dept. of Pharmacology & Therapeutics, The University of B. C., Vancouver, B. C., V6T1W5, Canada.

The properties of voltage-dependent potassium channels in cultured rat hypothalamic neurons have been studied using the patch clamp technique. With the inside-out mode and 140 mM K^+ in the bath and 5 mM K^+ in the pipette a channel with conductance of 52 pS is commonly observed after depolarization steps. This channel is selective for K^+ since the extrapolated reversal potential is close to that predicted for K^+ and shifts to a value of zero when the bath solution contains 5 mM K^+; the channel was non-conducting when the solution contained 140 mM Cs^+. Single exponential fits were adequate to describe the histograms of open times for the K^+ channels with membrane depolarization acting to increase the channel open time. The distributions of closed times required two-component fits reflecting short closed sojourns during open events and longer closed periods between openings. A concentration of 10 mM TEA, applied to the outside of the cell membrane (outside-out mode), blocked the 52 pS channel. In the cell-attached mode with no TTX in the solutions, outward K^+ currents were associated with the after-hyperpolarization phase of the capacitively recorded action potential. The properties of the K^+ channels studied are consistent with those associated with the delayed rectifier K^+ conductance and would serve to mediate electrical excitability in hypothalamic neurons.
(Supported by B. C. Health Care Research Foundation.)

484.4

REPETITIVE FIRING IN HIPPOCAMPAL NEURONS IS MODULATED BY THE MEMBRANE POTENTIAL PRIOR TO ACTIVATION, THROUGH THE ACTION OF A SLOWLY INACTIVATING POTASSIUM CURRENT, I_D. J. F. Storm, Institute of Neurophysiology, University of Oslo, Oslo 1, Norway.

In hippocampal neurons, the firing frequency in response to depolarizing current is regulated by spike-activated K currents (I_{AHP}, I_C, I_M). In addition to this "feedback" discharge control, there may be a "feed-forward" control by the membrane potential prior to activation of the cell (MP). Thus, both I_A and I_M are expected to modulate the initial firing, in ways which depend on their degree of activation (I_M) or inactivation (I_A) at rest. Here I report evidence that a third potassium current, I_D, mediates a particularly long-lasting influence by the MP on subsequent discharge. Voltage clamp measurements indicate that I_D inactivates more slowly and is more sensitive to 4-aminopyridine (4-AP) than I_A, but it activates faster than I_M or I_K (Storm, 1988, Biophys. J. 53: 148A).

CA1 pyramidal cells (n=36) in rat hippocampal slices, were impaled with KCl-filled micro-electrodes, at 31-35°C. Repetitive firing was elicited by injecting long depolarizing current pulses (1-10 s). The MP was controlled by steady current injection. At MPs close to the normal resting potential, there was often a 200-800ms delay to the onset of firing, while the cell depolarized slowly, forming a "ramp". The ramp and delay appeared to reflect inactivation of I_D because: (1) the input resistance increased during the ramp; (2) the ramp was blocked by 50 µM 4-AP; (3) hyperpolarization of the MP (to enhance I_D) increased the delay (up to 10s); whereas (4) depolarization (which inactivates I_D) abolished it. I_D also appeared to counteract the spike frequency adaptation, which was reduced by hyperpolarization and enhanced by depolarization. Plots of firing frequency against current intensity (f/I-plots), normally show a low-gain range ("primary range") for small intensities. This range was increased by hyperpolarization and abolished by depolarization of the MP, suggesting that I_D is essential for this low-gain part of the f/I relation.
(Supported by the Norwegian Research Council, NAVF).

484.5

PHENCYCLIDINE BLOCKADE OF VOLTAGE-DEPENDENT POTASSIUM AND CALCIUM CURRENTS IN ISOLATED HIPPOCAMPAL NEURONS: PHARMACOLOGY AND VOLTAGE-DEPENDENCY. J.M.H. ffrench-Mullen, M.A. Rogawski and J.L. Barker, Laboratory of Neurophysiology and Medical Neurology Branch, NINCDS, NIH, Bethesda, MD 20892.

Using whole-cell voltage clamp recording techniques, we previously observed that phencyclidine (PCP) produces a selective block of the sustained, voltage-dependent K^+ current (I_K) in hippocampal neurons (*Neurosci. Lett.*, in press). In the present study, we compared the K^+ channel blocking activity of PCP with that of a series of PCP and *sigma* recognition site ligands. In addition, we examined the effect of these compounds on Ca^{2+} currents. The IC_{50}'s (*uM*) for blockade of I_K were: PCP, 36; dexoxadrol, 73; levoxadrol, 260; (+)-SKF-10,047, >300; (-)-SKF-10,047, >300; (+)-3-PPP, >300. In contrast, the IC_{50}'s for blockade of the transient K^+ current (I_A) were: PCP, 330; dexoxadrol, 285; levoxadrol, 285. The blockade of I_K by PCP was voltage-dependent so that the fractional block decreased as the cell was depolarized. Assuming that PCP blocks I_K by entering the channel pore, calculations according to the method of Woodhull suggested that the binding site is about 5-10% into the electrostatic field. These experiments confirm that PCP (and also dexoxadrol/levoxadrol) selectively block I_K; I_A is only affected at very high concentrations of the drugs. Blockade of I_K by PCP occurs at a site that is pharmacologically similar to the "low affinity PCP" ([^{3}H]PCP-3-OH) binding site, but dissimilar to "high affinity PCP" or *sigma* binding sites (Itzhak, *Life. Sci.* 42:745-752, 1988). PCP also reduced Ca^{2+} current in these cells (IC_{50}, 10 *uM*) whereas (+)-SKF-10,047 and (+)-3-PPP were inactive (10 *uM*), suggesting that blockade of I_K and I_{Ca} may occur at pharmacologically similar sites.

484.6

CATION AND ANION PERMEABILITY DURING THE EXCITATORY RESPONSE TO ATP IN CHICK SKELETAL MUSCLE. S. A. Thomas and R. I. Hume. Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109

Adenosine 5'-triphosphate (ATP) has recently been shown to elicit two distinct currents in embryonic chick skeletal muscle (Hume and Thomas, Soc Neurosci Abstr 13:790); a rapidly activating, desensitizing current which has a reversal potential near -15 mV, and a more slowly activating current that reverses at the potassium equilibrium potential. The late current is due to an increase in the permeability to potassium, but the early current might result from an increase in the permeability to various combinations of cations or anions. We performed ion substitution experiments to determine the ions that are permeable during the early response to ATP. We found that ATP increases membrane permeablility to anions as well as monovalent and some divalent cations, but not to larger ions like tetraethylammonium or glucuronate.

We determined the reversal potential of the early response to ATP in different external solutions, and used the Goldman equation to calculate permeability ratios. There was little difference in the permeability to the monovalent cations sodium, potassium and cesium. On the other hand, larger cations like tetraethylammonium were much less permeable. In addition, the divalent cations magnesium, calcium, and barium (but not cobalt) were significantly permeable. However, isotonic concentrations of these divalent cations, and much lower (5 mM) concentrations of manganese, zinc, and cadmium blocked the response to ATP. Finally, two independent lines of evidence indicated that small anions such as chloride and acetate were also permeable, while the larger anion glucuronate was much less so. We are now testing the possibility that the cation and anion permeability may be through a single charge nonselective ion channel activated by ATP.

484.7

A CALCIUM-DEPENDENT, VOLTAGE SENSITIVE CHLORIDE CURRENT IN CHICK SKELETAL MUSCLE. R. I. Hume and S. A. Thomas. Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109

Skeletal muscle of many species is known to possess regenerative sodium and calcium spikes. Unlike several other species, however, developing chick skeletal muscle also possesses a long-lasting afterdepolarization (AD). We have confirmed reports that the AD is the result of a chloride current, and demonstrate that activation of this current is calcium-dependent and voltage sensitive.

A large positive shift in the peak potential of the AD occurred when external chloride was replaced by glucuronate. However, the AD disappeared when external calcium was lowered from 1 to 0.1 mM. Replacing external calcium with barium resulted in barium spikes following depolarization, but no AD. When cobalt replaced calcium, no regenerative responses were present. In these experiments, the sodium spike was blocked with tetrodotoxin. With external cobalt but no calcium, the AD could often be restored by including 20 mM calcium in the 3 M KCl recording electrode. This effect could not be mimicked with magnesium.

Whole cell patch clamp recordings were made from myoblasts under conditions in which chloride was the only plausible charge carrier. Large currents were observed following depolarization in approximately half the cells when internal calcium was buffered at 10^{-6} M, but currents were absent or greatly attenuated at 10^{-8} M. The reversal potential for these currents depended on E_{Cl} and activation and inactivation were both voltage sensitive.

484.8

THREE TYPES OF CHLORIDE CHANNELS IN CULTURED RAT HIPPOCAMPAL NEURONS

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Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892 and *Dept. of Pharmacology, University College London, London WC1, U.K.

We have recorded from inside-out patches of somatic membrane from cultured hippocampal neurons, in order to isolate ion channels that might underlie slow depolarization-activated macroscopic tail currents recorded from these cells. We initially recorded from patches using asymmetric NaCl solutions in order to detect both cation and anion channels. Reversal potentials were determined using voltage ramps applied to the patch. Ca^{2+}-activated channels were sought by applying Ca^{2+} (10nM - 10μM) to the cytosolic face of patches. Cation-selective channels proved extremely rare, whereas anion-selective channels were observed frequently. Solutions containing the impermeant cation N-methyl D-glucamine (NMG) and Cl^- ions were used to isolate anion channels. Improved seals (250 GΩ) and reduced background noise were obtained under these conditions, and it was possible to resolve three types of anion channels quite readily, none of which was identical with GABA-activated anion channels: (1) a "big" voltage-activated channel (B_{Cl}), activated at about -25mV by depolarizing ramps from -50mV (whole cell convention). The B_{Cl} channel was only slightly selective for Cl^- over Na^+ or NMG^+, and exhibited a main conductance state of ~300pS. (2) A Ca^{2+}-activated channel (Cl_{Ca}; ~20pS) seen at $[Ca^{2+}]_i > 0.5\mu M$, which was voltage-insensitive. (3) A 'leak' channel (Cl_L; ~8-12pS), which was Ca^{2+}- and voltage-insensitive. Both Cl_L and Cl_{Ca} channels were highly selective for Cl^- over Na^+ or NMG^+. The macroscopic current generated by Cl_L channels may be a factor in the resting conductance of these cells. The relationship between B_{Cl} and Cl_{Ca} and whole cell currents awaits further investigation.

484.9

THE VOLTAGE DEPENDENCE OF ACTIVATION BY CALCIUM OF CHLORIDE CHANNELS IN XENOPUS OOCYTES. E.M. Landau, B. Gillo and T. M. Moriarity. Depts. of Psychiatry and Pharmacology, Mt. Sinai School of Medicine and the Bronx VA Medical Center, New York, NY.

The voltage sensitivity of the calcium-dependent chloride channels in the oocyte derives from the voltage dependence of the plasma membrane calcium channels. We now report that the voltage sensitivity of chloride channel activation was altered dramatically when intracellular calcium was increased independently of changes in the membrane potential. This was achieved by treating the cells with cadmium (0.2-0.4 mM), by injecting them with inositol-trisphosphate (0.2-0.4 pmol) or by exposing them to the calcium ionophore A23187 in the presence of calcium. Under these circumstances the chloride current occurred at voltages between -100 and +100 mV and the conductance increased ten-fold per 150-210 mV depolarization in the range of -100 to -10 mV. At more positive voltages the conductance appeared to saturate. It is concluded that intracellular calcium activates the chloride channel by acting on a site located part of the way through the membrane thickness.

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484.10

CHIRAL CLOFIBRIC ACID ANALOGS: R-(+) OPEN, AND S-(-) BLOCK CHLORIDE CHANNELS IN RAT SKELETAL MUSCLE. D.Conte-Camerino*, S.H.Bryant, M.Mambrini*, A.DeLuca*, D.Tricarico*, V.Tortorella*, and G.Bettoni*. Med. Chem. and Pharmacobiology Departments, University of Bari, 70126-Bari, Italy and Department of Pharmacology and Cell Biophysics, University of Cincinnati, Cincinnati, OH 45267.

Enantiomeric pairs of four 2-(p-chlorophenoxy)isobutyric acid (clofibric acid or CPIB) analogs were synthesized, resolved and the absolute configurations determined. The analogs used were the methyl, phenyl, propyl and hexyl substitutions on the chiral alpha carbon. Measurements of chloride conductance, membrane potentials and membrane excitability were made on rat extensor digitorum fibers in vitro at 30°C with standard two microelectrode methodology. The S-(-) isomers of each analog produced only a block of chloride conductance with increasing concentrations until 100% block. The R-(+) isomers, on the other hand, increased chloride conductance by as much as 17% to 39% at low concentrations, but at higher concentrations decreased chloride conductance, but never by more than 25% of the control value. Racemic mixtures produced only a chloride conductance block. The conductance increase effect is antagonized by the S-(-) isomers. The actions of the enantiomeric pairs to either produce or inhibit myotonic excitability paralleled their ability to block or increase chloride conductance, respectively. (Supported by NIH Grant NS-03178 and an MDA Grant-in-aid.)

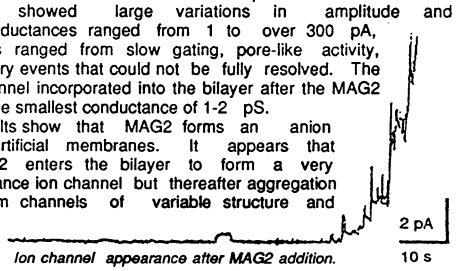
484.11

MAGAININ 2, AN ANTIBIOTIC FROM THE AFRICAN CLAWED FROG INDUCES SINGLE CHANNEL ACTIVITY IN ARTIFICIAL MEMBRANES. R.A. Cruciani[1]*, J.L. Barker[1], M. Zasloff[2]* and E.F. Stanley[3], [1]LNP and [3]LB, NINCDS; [2]HG, NICHD, NIH. Bethesda MD 20892.

Magainin 2 (MAG2), a 23 amino acid peptide from the skin of *Xenopus Laevis*, exhibits antimicrobial activity against a broad range of microorganisms (Zasloff, PNAS 84:5449). We have previously shown that MAG2 increases the anion conductance of artificial lipid bilayers (Cruciani et al., Bioph. J. 53:9a). In this study we have examined the single channel activity of MAG2.

Lipid bilayers of PS-PC in n-decane were formed on the tip of 3 to 8 μm micropipettes in a symmetrical 600 mM KCl solution. Increases in bilayer conductance were observed after addition of MAG2 to the external solution. This conductance was composed of discrete current steps that showed large variations in amplitude and kinetics. Conductances ranged from 1 to over 300 pA, and kinetics ranged from slow gating, pore-like activity, to fast flickery events that could not be fully resolved. The first ion channel incorporated into the bilayer after the MAG2 addition had the smallest conductance of 1-2 pS.

These results show that MAG2 forms an anion channel in artificial membranes. It appears that initially MAG2 enters the bilayer to form a very small conductance ion channel but thereafter aggregation occurs to form channels of variable structure and stability.



Ion channel appearance after MAG2 addition.

485.1

TWO DIFFERENT G-PROTEINS MAY MEDIATE RECEPTOR-STIMULATED PHOSPHOLIPID BREAKDOWN IN CULTURED RAT SENSORY NEURONS, Teresa M. Perney* and Richard J. Miller (SPON: M.E. Gurney). Dept. of Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Recent evidence has suggested that the metabolism of phosphatidyl inositol 4,5,-bisphosphate (PIP_2) is regulated by a G-protein. However, the identity of the G-protein involved is still uncertain. We investigated the ability of neuropeptide Y (NPY) and bradykinin (BK) to stimulate the hydrolysis of PIP_2 in cultured dorsal root ganglion (DRG) neurons and the sensitivity of both responses to pertussis toxin (PTx) and cholera toxin (CTx). We observed that both NPY and BK stimulated a transient release of [^{3}H]inositol trisphosphate ([^{3}H]IP_3) from [^{3}H]inositol prelabeled DRG neurons. NPY (EC_{50} = 5 nM) stimulated a 2-5 fold increase in [^{3}H]IP_3 levels which peaked at 30 sec, while BK (EC_{50} = 2 nM) produced a 10-15 fold increase in [^{3}H]IP_3 within 15 sec. The effects of BK and NPY on [^{3}H]IP_3 are additive. In addition, both NPY and BK stimulated [^{3}H]IP_3 formation were subject to negative feedback inhibition by short-term treatment with phorbol esters. When DRG neurons are pretreated with PTx (350 ng/ml) the ability of NPY to stimulate [^{3}H]IP_3 production was abolished, while the response to BK was unaffected. Furthermore, pretreatment of DRG neurons with CTx (2 μg/ml) enhanced only the NPY stimulated formation of [^{3}H]IP_3. Microspectrofluorometric measurements demonstrated that both NPY and BK were able to increase $[Ca^{2+}]_i$ in the same fura-2 loaded neuron. The increase occured in Ca^{2+}-free media indicating that the IP_3 produced was able to release intracellular Ca^{2+} stores. These results suggest that different G-proteins can mediate receptor-stimulated PIP_2 hydrolysis and Ca^{2+} mobilization in the same cell.

485.2

THE EFFECTS OF KINASE C ACTIVATING AND INHIBITING AGENTS ON MEMBRANE PROPERTIES OF RAT HIPPOCAMPUS IN VITRO. N. AGOPYAN and K. KRNJEVIC, Depts. Physiol. and Anaesthesia Research, McGill University, Montréal, Québec, Canada.

In previous experiments on hippocampus in situ both activation [by phorbol diacetate (PDAc)] and inhibition [by 1-2-methylpiperazine dihydrochloride (H-7)] of kinase C resulted in enhancement of population spikes (Agopyan and Krnjević 1987, Soc. Neurosci. Abst. 13:64). We have now studied the effects of PDAc and H-7 in vitro.

Hippocampal slices were maintained in an interface chamber and intracellular recordings were made from the cell bodies of CA1 pyramidal cells.

Iontophoretic application of PDAc increased the amplitude of both EPSPs and IPSPs, reduced the number of spikes evoked by depolarizing pulses and the membrane resistance, without affecting the membrane potential. Iontophoresis of H-7 on the other hand increased the amplitude and the number of action potentials induced by both orthodromic and antidromic stimulation, and reduced IPSPs and the membrane resistance.

These results, in keeping with those obtained in situ, suggest that kinase C activation enhances both EPSPs and IPSPs, whereas inhibition of kinase C leads to depression of IPSPs.

Supported by SAVOY Foundation and MRC.

485.3

MUSCARINIC ACTIVATION OF IP_3 MEDIATED CHLORIDE CURRENT IS INHIBITED BY THE βγ-SUBUNITS OF G-PROTEINS. T.M. Moriarty*, B. Gillo*, D. Carty*, R. Premont*, R. Iyengar* and E.M. Landau (SPON: J. Thornborough). Depts. of Psychiatry and Pharmacology, Mt. Sinai School of Medicine and Bronx V.A. Medical Center, New York, N.Y.

The Xenopus oocyte has a muscarinic (MAch) activated Cl- current which is mediated by IP3 and is probably transduced by a G-protein. This study examines the mechanism of G-protein coupling of receptors to IP3 production. The MAch response was examined in two-electrode voltage clamped oocytes in a superfusion apparatus. Modulation of the MAch evoked Cl- current was examined in oocytes microinjected with resolved G-protein subunits purified from bovine brain and human RBC. The presence of a G-protein in oocytes was shown by pertussis toxin (PTX) labeling of a 40 kDa band from oocyte membranes. A 36 kDa band was labeled by an antiserum specific for the β-subunit. PTX treatment uncoupled the MAch receptors from activation of the Cl- current. Cells microinjected with 1.5 ng of βγ showed a 95% reduction in the MAch activated Cl- current. Cells injected with equal volumes of βγ storage buffer showed no change in response. Cells injected with boiled βγ, bovine serum albumin or resolved α-subunits also showed no reduction in response. Cells injected with varying concentrations of βγ showed a dose-response relationship with half-maximal inhibition of MAch activated Cl- current at about 1 nM. Cells injected with 1.5 ng βγ could not response to bath applied agonist, but could generate the Cl- current on intracellular injection of IP3. These results suggest that there is a G-protein responsible for MAch receptor mediated signal transduction through IP3, that it is an αβγ heterotrimer and that its mode of action is similar to that of the hormone activated adenylyl cyclase.

485.4

MOLECULAR ISOLATION OF A PUTATIVE PHOTORECEPTOR-SPECIFIC PHOSPHOLIPASE C GENE OF DROSOPHILA, norpA, AND ITS ROLE IN PHOTOTRANSDUCTION. **B.T. Bloomquist*, R. Shortridge*, S. Schneuwly*, M. Perdew*, and W.L. Pak. Dept. of Biological Sciences, Purdue Univ., W. Lafayette, IN 47907**

Severe norpA mutations of Drosophila eliminate the photoreceptor potential. Evidence suggests that the norpA gene encodes a protein involved in an intermediate step of phototransduction. Inoue et al. (1985) and Selinger and Minke (1988) have reported that norpA mutations affect the phospholipase C (PLC) activity in the eye. We obtained from C. Montell, H. Steller, and G. Rubin two cosmids containing DNA from the region between the distal breakpoints of two gamma-ray induced deficiencies, Df(1)rb4 and Df(1)biD2, which define the location of the norpA gene. To prove that the gene is contained in the cloned DNA and to localize it, we isolated in transposon mutagenesis a norpA allele which contains a hobo element in the same region as that cloned into the cosmids. Northern hybridizations show that the putative norpA transcript is approx. 7 kb in length and expressed only in the adult head. In situ hybridizations of a cDNA corresponding to this region to adult tissue sections show heaviest labels in photoreceptors. Conceptual translation of an open reading frame contained in cDNAs corresponding to this region generates a large protein with extensive sequence similarity to the only published eukaryotic PLC sequence (Stahl et al., 1988). We conclude: (1) the norpA gene encodes a PLC; (2) the PLC is a critical component of phototransduction machinery in Drosophila.

485.5

CHARACTERIZATION OF THE SOLUBILIZED $INSP_4$ BINDING SITE IN RAT CEREBELLUM. P.B. Sklar, A.B. Theibert* S. Supattapone, R. Mourey*and S.H. Snyder. The Johns Hopkins University Sch. of Med., Dept. of Neuroscience, Baltimore, MD 21205.

Inositol 1,3,4,5-tetrakisphosphate ($InsP_4$) is produced rapidly and transiently upon stimulation of the phosphoinositide system. In several reported studies microinjection of $InsP_4$ in conjunction with inositol 1,4,5-trisphosphate ($InsP_3$) mimicked receptor activation, suggesting a role for $InsP_4$ as a second messenger in signal transduction.

We have demonstrated specific binding sites for [^{3}H]$InsP_4$ in rat cerebellar membranes. Recently we have detergent solubilized and partially purified this cerebellar $InsP_4$ binding site. Specific activity of binding is increased approximately 100-fold over homogenates. The binding site is separated, during the purification, from the $InsP_3$ receptor, the $InsP_3$/$InsP_4$ 5-phosphatase, and the $InsP_3$ 3-kinase. Characterization of this [^{3}H]$InsP_4$ binding reveals an IC_{50} for $InsP_4$ of 80 nM. $InsP_3$ and $InsP_2$ are much weaker at competing binding, both showing IC_{50}'s > 1 μM. Binding is potently inhibited by heparin and calmodulin. Finally, solubilized [^{3}H]$InsP_4$ binding is observed in several peripheral tissues, including those of the immune system.

485.6

MOLECULAR ANALYSIS OF REGULATORY SUBUNITS OF cAMP-DEPENDENT PROTEIN KINASE (PKA) IN *APLYSIA* NEURONS. P.J. Bergold, S. Beushausen, S. Sturner*, H. Bayley, and J. H. Schwartz. Center for Neurobiology and Behavior and Howard Hughes Medical Institute, Columbia University, 722 W. 168th St., New York, NY 10032 and Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545

cAMP, through activation of PKA, is a critical mediator of the changes in *Aplysia* neurons that occur during both short- and long-term sensitization. It has been proposed that a sustained increase in the activity of PKA is an essential difference between short- and long-term sensitized cells. Changes in the ratio of the two types of PKA subunits is one mechanism for altering the activity of the enzyme. We have previously shown that the level of the regulatory subunits (R) decrease as compared to the catalytic subunit (C) during long-term sensitization as measured with the cAMP-photoaffinity reagent, [^{32}P] 8-N_3-cAMP. To study the specific roles the neuronal isoforms of PKA have in mediating synaptic plasticity, we have isolated genomic and cDNA clones encoding R subunits. Partial sequence analysis of a genomic clone isolated by low stringency hybridization to a bovine R_I cDNA (provided by Dr. G. S. McKnight, U. of Washington) revealed two exons with greater than 90% predicted amino acid identity with bovine R_I. An oligonucleotide probe derived from the sequence of one of these exons was used to screen an *Aplysia* neuronal cDNA library. One positive phage was isolated from approximately 3 X 10^6 recombinants. The 3.5 kb insert of this clone contained sequence encoding the C-terminal 234 amino acids of R_I including the translation terminator. The sequence predicts a protein with greater than 80% amino acid identity with bovine R_I. Using the *Aplysia* R_I probe, a rare transcript of 8 kb was detected on Northern blots of neuronal polyA+ RNA. Several polyclonal antisera were prepared against a peptide predicted by the genomic DNA sequence to be part of the *Aplysia* R_I cAMP-binding domain. One of these antisera reacted strongly with a M_r 54,000 neuronal protein on Western blots. We can now measure the changes in PKA that occur in *Aplysia* sensory neurons during the induction of long-term sensitization with these reagents for R together with those for C described by Beushausen et. al., (These Abstracts).

485.7

TWO ISOFORMS OF THE cAMP- DEPENDENT PROTEIN KINASE (PKA) CATALYTIC SUBUNIT IN APLYSIA NEURONS ARISE THROUGH ALTERNATIVE SPLICING S. Beushausen, P. J. Bergold, S. Sturner*, A. Elste*, V. Roytenberg*, J. H. Schwartz and H. P. Bayley. Howard Hughes Medical Institute, Center for Neurobiology and Behavior, Columbia University, 722 W 168th St., NY, NY, 10032. Worcester Foundation for Experimental Biology, 222 Maple Ave, Shrewsbury, MA 01545.

Studies in Aplysia have implicated cAMP-dependent protein kinase in several instances of synaptic plasticity including short and long term facilitation in sensory neurons. To assist in the analyses of these processes we have isolated genomic and cDNA clones encoding catalytic (C) subunits of the enzyme.

A randomly primed Aplysia neural cDNA library was screened with a mouse Cα cDNA (provided by G.S. McKnight). Nucleic acid sequence analysis of the clones revealed two different classes of cDNA encoding putative C isoforms. Both contained open reading frames 1056 nucleotides in length, but differed at 39 positions between residues 423-549. The inferred amino acid sequences indicated that the isoforms were 97% identical, differing at 10/42 positions between residues 142-183. The Aplysia amino acid sequences were 83-85% identical to murine and bovine Cα and Cβ, and 83% identical to the Drosophila subunit. Sequence analysis of an Aplysia genomic DNA fragment and S1 nuclease mapping of cellular RNA confirmed that this variation arises through alternative splicing of two mutually exclusive exon cassettes with homology and equal length to exon 6 of the mouse Cα gene. In contrast, S1 analysis of mouse brain and liver RNA using a Cα probe failed to show alternative splicing in this region. Although not strictly tissue specific, the Aplysia transcripts were expressed at different relative levels in different tissues. For example, the major transcript in neurons was present at relatively low levels in ovotestis.

Recently, clones for the regulatory (R) subunits of the kinase have been obtained (Bergold et al., These Abstracts). Next, we intend to use nucleic acid and immunologic probes to investigate how combinatorial expression of R and C isoforms might be involved in synaptic plasticity.

485.8

G_S ACTIVATES ADENYLATE CYCLASE IN NEOSTRIATAL PRESYNAPTIC TERMINALS. N. Aronin. Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01655

G_S is a GTP-binding protein which mediates receptor activation of adenylate cyclase. Here the presynaptic activation of adenylate cyclase by G_S was examined in rat neostriatal synaptosomes. First, since $G_S\alpha$ is ribosylated specifically by cholera toxin (CT), neostriatal synaptosomes were incubated in a medium containing ^{32}P-NAD and activated CT (10 µg/ml). ^{32}P-ribosylated proteins were then separated by 10% SDS-polyacrylamide gel electrophoresis. Second, synaptosomes were treated with activators of $G_S\alpha$, NaFl (10mM) and CT (10 µg/ml), and the generated cAMP was measured by radioimmunoassay. Results: After ^{32}P-NAD ribosylation of synaptosomal proteins by CT, two major bands were identified, at 45k and 52k, consistent with previously reported molecular weights of $G_S\alpha$. Labeling of proteins was absent in the synaptosomes when CT was omitted. Cholera toxin and NaFl increased cAMP generation in the synaptosomes by 200% and 400%, respectively. By electron microsopic examination, the synaptosomal preparation reproducibly contained about 90% synaptosomes without postsynaptic elements. These findings indicate that in the synaptosomes $G_S\alpha$ is linked to adenylate cyclase activation. Since axoaxonal synapses are rare in the neostriatum and G_S is generally thought to transduce signals of membrane-bound receptors, these results point to paracrine, autocrine or hormonal regulation of adenylate cyclase activity in presynaptic terminals in the mammalian neostriatum.

485.9

EVIDENCE THAT LIPOXYGENASE METABOLITES LINK DOPAMINE D2 RECEPTORS TO POTASSIUM S-CHANNELS IN APLYSIA SENSORY NEURONS. Y. Yaari. Howard Hughes Med. Inst., Columbia Univ. Col. of Phys & Surg., New York, NY 10032.

Dopamine (DA) is a putative inhibitory transmitter in some synapses in Aplysia. When applied onto Alysia pleural sensory neurons in culture, it produced membrane hyperpolarization and conductance increase by activating the potassium S-current. While acting through distinct D2-like receptors (as indicated by the effects of selective D1 and D2 agonists and antagonists), DA mimicked the inhibitory action of the peptide transmitter FMRFamide, which is thought to be mediated by the production of lipoxygenase metabolites of arachidonic acid. DA may stimulate the same cascade, as (i) inhibition of phospholipase activity with p-bromophenacyl-bromide prevented the actions of DA and FMRFamide; (ii) blockage of the lipoxygenase pathway with nordihydroguaiaretic acid markedly reduced the actions of both transmitters, while (iii) blockage of the cycloxygenase pathway with indomethacin had no effect; (iv) DA exerted its action in neurons loaded with cyclic-AMP analogues, indicating that it does not act by inhibiting adenylate cyclase.

Thus D2-like receptors mediating inhibition in Aplysia sensory neurons may be linked to potassium S-channels by lipoxygenase metabolites of arachidonic acid.

485.10

FORMATION OF THE 12-LIPOXYGENASE PRODUCT 12-KETO-EICOSATETRAENOIC ACID (12-KETE), A POSSIBLE SECOND MESSENGER IN APLYSIA NEURONS. D. Piomelli, E. Shapiro, S. Feinmark* and J.H. Schwartz, HHMI and Dept. of Pharmacology, Columbia Univ. Col. of Phys. & Surg., New York, NY 10032.

The 12-lipoxygenase product 12(S)-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12-HPETE) mimics synaptic responses produced by histamine and Phe-Met-Arg-Phe-amide (FMRF) in identified neurons of the marine mollusk, Aplysia. Since 12-HPETE is known to be metabolized further in other animals, we investigated whether these actions might require conversion of 12-HPETE to other active products. Here we report the identification of 12-keto-5,8,10,14-eicosatetraenoic acid (12-KETE), a metabolite of 12-HPETE formed by Aplysia nervous tissue. Release of [^{3}H]12-KETE occurs when the nervous tissue is labeled by incubation with [^{3}H]- arachidonic acid and then stimulated with histamine. In L14 cells, a cluster of identified motor neurons that produce inking, application of 12-HPETE elicits a change in membrane potential similar to that evoked by histamine. A similar effect is produced by 12-KETE provided by A.R. Brash (Vanderbilt U) but not by 12(S)-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE), another metabolite of 12-HPETE, suggesting that 12-HPETE and its metabolite 12-KETE participate in the transduction of histamine responses in Aplysia neurons.

485.11

CALCIUM DEPENDENT EFFECTS OF MAITOTOXIN ON PHOSPHOINOSITIDE BREAKDOWN AND ON CYCLIC AMP ACCUMULATION IN PC12 AND NCB-20 CELLS. Fabian Gusovsky, Takeshi Yasumoto* and John W. Daly. LBC, NIDDK, NIH, Bethesda, MD 20892.

The marine dinoflagellate toxin maitotoxin (MTX) stimulates phosphoinositide breakdown in pheochromocytoma PC12 cells and in neuroblastoma hybrid NCB-20 cells in a calcium dependent, calcium channel blocker resistant fashion. In PC12 cells the maximal stimulation of phosphoinositide breakdown occurs at 1.5 mM $[Ca^{2+}]_o$, whereas in NCB-20 cells the maximal stimulation is observed at 2.5-4.5 mM $[Ca^{2+}]_o$. Phosphoinositide breakdown leads to both inositol phosphates and to diacylglycerides. The latter through stimulation of protein kinase C would, like phorbol esters, be expected to augment cyclic AMP accumulation in PC12 cells and to inhibit receptor-mediated cyclic AMP accumulation in NCB-20 cells. MTX did potentiate forskolin-induced accumulation of cyclic AMP in PC12 cells and did inhibit prostaglandin E_2-induced accumulation of cyclic AMP in NCB-20 cells. The effects of MTX on accumulation of cyclic AMP were calcium-dependent and the concentrations of calcium required for maximal responses were the same as the ones required for maximal stimulation of phosphoinositide breakdown. The results confirm previous studies on the heterogeneous input of protein kinase C to cyclic AMP-generating systems performed with phorbol esters and demonstrate the utility of MTX as a unique tool for studies of systems that involve second messengers generated through stimulation of phosphoinositide breakdown.

485.12

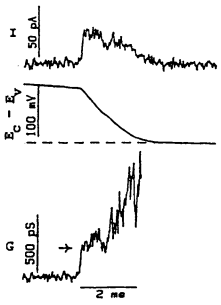
PHOSPHATIDYLCHOLINE TURNOVER AS A REPORTER FOR THE ACTIVATION OF DISTINCT CELLULAR SIGNALLING PATHWAYS: IMPLICATIONS FOR THE ACTION OF NGF AND CAPSAICIN. S.I. Patterson+,* A. Gatti* and M.R. Hanley . +Dept Neurobiology, Stanford University School of Medicine, Stanford, Ca 94305, USA and MRC Molecular Neurobiology, Hills Rd, Cambridge CB2 2QH, UK.

Turnover of membrane phosphatidylcholine (PC) shows both constitutive and pharmacologically-stimulated components in a neuronal cell line NG115-401L which can be followed by the extracellular release of radioactive choline metabolites. Although independently regulated, both constitutive and stimulated release occur through phospholipase D, releasing ^{3}H-choline into the medium. The basal release is reduced by agents that raise intracellular calcium, and by cAMP stimulants or mimetics. These agents do not influence a second class of stimulated PC turnover effects which are sensitive to protein kinase C activators, and can be down-regulated by persistent exposure to phorbol diesters. Moreover, the detailed pharmacology of the stimulated component indicates that NGF and capsaicin may be additional and independent stimulants as they are additive with phorbol diester stimulation of turnover. These results suggest that both NGF and capsaicin may activate novel or known kinases early in their signalling pathways. The hormone bradykinin, which causes both intracellular calcium mobilization and PKC activation in these cells, shows simultaneous regulation of these two distinct processes.

486.1

TIME COURSE OF FUSION PORE CONDUCTANCE DURING EXOCYTOSIS OF MAST CELL SECRETORY VESICLES. A. Spruce,* L. Breckenridge,* W. Almers, Dept. Physiol., U. Washington, Seattle, WA 98195

Exocytosis begins with the opening of a fusion pore that connects vesicle lumen with cell exterior (Breckenridge & Almers, Nature 328:814). When the pore first opens, a transient outward current through it (trace I) equalizes the potentials across vesicle (E_V) and cell membranes (E_C). ($E_C - E_V$) is obtained by subtracting the time integral of I from its final value and then dividing by the vesicle capacitance. The pore conductance (trace G) is given by $I/(E_C - E_V)$. G rises abruptly to a value G_o (arrow); the rise time (102±45 μs SD at 10 kHz) is not well resolved. Later, G grows more gradually as the pore dilates. Sometimes (12% of cases) the slow phase is too rapid to be distinguished from the fast. The histogram of G_o is skewed (range 80-800 pS, peak 220 pS, median 270 pS, 195 transients). The abrupt opening of the pore, faster than in gap junction channels (Neyton & Trautman, J Exp Biol 124:93; Veenstra & deHaan, Am J Physiol 254:H170), is reminiscent of the abrupt opening of single ion channels. NIH grants AR17803 and GM 39520.



486.2

TIME COURSE OF TRANSMITTER RELEASE AND CALCIUM CHANNELS AT THE MOUSE MOTOR NERVE TERMINAL. A. I. Bain* and D. M. J. Quastel. Dept of Pharmacology & Therapeutics, Univ. of British Columbia, Vancouver, B. C., Canada V6T 1W5

Following nerve stimulation the quantal components of EPPs are sharply focused in time with most appearing in a period of about 0.4 ms. Using a computer to locate each quantum and minEPP, we find that changes in release rate and latency produced by repetitive stimulation in the presence of Ba are consistent with a fixed underlying time course of intracellular Ca/Ba at release sites, with time constants of about 0.5 ms for the rising and falling phases. Brief submaximal focal depolarizations of nerve terminals (NTDs) elicit quanta with a more dispersed latency. The effectiveness of NTD trains to produce rise of minEPP frequency in the presence of Ba provides a method for assessing the relative extent of Ca-channel opening by pulses; results show no substantial channel inactivation up to at least 100 ms and no inhibition of ion entry except by pulses much larger than those causing maximal activation. By extrapolation from results with varied Ca/Ba it appears that a component of the EPP should persist even in the absence of ion entry and, with 'buffered' Ca or with ion entry inhibited by bekanamycin there indeed occur small EPPs that grow with high frequency stimulation, in proportion to minEPP frequency. Thus, nerve impulses influence release by a mechanism(s) other than Ca channel gating.

(Supported by Muscular Dystrophy Assoc. and MRC, Canada)

486.3

ROLES OF PRESYNAPTIC CALCIUM AND POTENTIAL IN RELEASING NEUROTRANSMITTER. R.S. Zucker and P.G. Haydon. Physiol.-Anat. Dept., Univ. Calif., Berkeley, CA 94720 and Zool. Dept., Iowa State Univ., Ames, IA 50011.

We have examined the roles of presynaptic membrane potential and intracellular calcium activity in triggering the release of acetylcholine at an inhibitory synapse formed between two isolated somata from the snail Helisoma trivolvis. Buccal ganglion neurons B5 and B19 were co-cultured under conditions which suppress neurite outgrowth and permit formation of a soma-soma synapse. Synaptic transmission was monitored as miniature inhibitory postsynaptic currents in cell B19 recorded using whole cell patch clamp. Presynaptic membrane potential was controlled by simultaneous whole cell patch clamp of neuron B5. Presynaptic internal calcium concentration was controlled by internal perfusion of B5 with the photodynamic chelator Nitr-5, 75% loaded with calcium. We observed: 1) Depolarization of B5 to above +15 mV releases transmitter in a normal-calcium medium. 2) Voltage-induced transmitter release is reduced by presynaptic introduction of Nitr-5, and abolished in a zero-calcium medium. 3) Photolysing Nitr-5 to raise presynaptic free calcium from 240 nM to over 1.5 μM elicited transmitter release. Release increased to about the rate caused by normal action potentials as calcium concentration rose to 10 μM, which is near the level occuring at release sites during action potentials. 4) Transmitter release was independent of presynaptic potential in the range -120 to +40 mV, when internal calcium was elevated by Nitr-5 photolysis and external calcium was eliminated. We conclude that action potentials normally release transmitter solely by admitting external calcium to release sites, with no direct effect on secretion. Supported by NIH Grants NS 15114 and NS 24233.

486.4

DIFFERENT FUNCTIONAL ROLES FOR TWO COMPONENTS OF CALCIUM CURRENT IN APLYSIA SENSORY NEURONS. B.W. Edmonds, E.R. Kandel and M. Klein*, Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, New York, NY 10032.

Calcium current was recorded in isolated Aplysia sensory cells using the whole-cell version of the patch-clamp technique. Currents obtained over a range of test voltages suggest the presence of two distinct components: a largely non-inactivating current which begins to activate around -20 mV and an inactivating current which is recruited at higher voltages (> 0 mV). A pharmacological separation has been achieved with nifedipine and FMRFamide. Nifedipine selectively antagonizes the non-inactivating component of current, whereas FMRFamide selectively reduces the inactivating component. Because FMRFamide is a transmitter mediating presynaptic inhibition in sensory cells, selective modulation of the inactivating component of calcium current may be important (in addition to enhancement of K^+ conductance) for the expression of this form of plasticity. Unlike FMRFamide, nifedipine does not inhibit transmitter release. Taken together, the data are consistent with the idea that of the two components of current, only the inactivating component is important for transmitter release and for at least one form of synaptic plasticity.

486.5

DOES FMRFamide-STIMULATED RELEASE OF ARACHIDONIC ACID DEPEND ON Na/H EXCHANGE OR INTERNAL CALCIUM? H. Blumenfeld*, B. Bug, N. Buttner*, J.D. Sweatt* and S.A. Siegelbaum. Ctr. for Neurobiol. & Behav., HHMI, Columbia Univ., New York, NY 10032

FMRFamide increases the S K^+ current in Aplysia sensory neurons through lipoxygenase metabolites of arachidonic acid (A.A.). Two mechanisms proposed to control release of A.A. through stimulation of phospholipase A_2 are an increase in Ca_i or an increase in pH_i due to Na/H exchange. Here we study possible roles for Ca_i or pH_i in the response to FMRFamide.

FMRFamide had no effect on resting Ca_i (100 nM), as determined by fluorescence ratios with fura-2 in the soma or growth cone. However, FMRFamide did decrease the Ca_i transient in response to action potentials in both cell body (23%) and growth cone (58%), consistent with its role in presynaptic inhibition. Next, the role of Na/H exchange was studied by replacing external Na with N-methyl-d-glucamine. Na removal reduced the S current response to FMRFamide by 66% but did not effect the response to A.A. Thus, the Na-sensitivity lies at a stage leading to A.A. release. However, pH_i (7.0) measured with BCEF showed no change in response to FMRFamide. FMRFamide also did not speed recovery from acidification following exposure to NH_3, arguing against marked stimulation of Na/H exchange. The inhibition of the FMRFamide response in Na-free medium could be related to a decrease of 0.3 pH_i units we see upon Na removal. Thus, the FMRFamide-mediated release of A.A. does not require an increase in Ca_i or pH_i.

486.6

PRESYNAPTIC CALCIUM AT MOTOR NERVE TERMINALS DURING POST-TETANIC POTENTIATION. K.R. Delaney*, D.W. Tank and R.S. Zucker (SPON: A. Dearry). Physiol.-Anat. Dept., Univ. Calif., Berkeley, CA 94720 and Molec. Biophys. Dept., AT&T Bell Labs., Murray Hill, NJ 07974.

We have measured calcium levels in individual presynaptic terminals of the excitor axon to the crayfish claw opener muscle while recording excitatory junctional potentials (EJPs). Fura-2 (17 mM in 100 mM KCl) was iontophoretically injected (15-20 nA for 30-60 min) into the preterminal axon, which was then stimulated extracellularly while EJPs were recorded in proximal fibers. Terminals (2-7 um dia.) filled with fura-2 on the same or an adjacent muscle fiber were imaged during stimulation using a cooled charge-coupled device camera attached to an upright microscope with a 40X objective. Long-lasting facilitation of the EJP was observed following tetanic stimulation of the excitor axon (9 of 10 preparations with 7-10 min 20-33 Hz tetani). Presynaptic free calcium concentration increased rapidly after the onset of stimulation and rose further during the tetanus, as did the EJP. Increased presynaptic calcium was closely correlated with potentiation of the EJP (tested at 1 Hz) during the first 5-10 min after the tetanus. In 5 of 7 preparations, a measureable enhancement of the EJP was observed more than 20 min after the tetanus, although calcium had returned to pre-stimulus levels. The results are consistent with a rise in presynaptic calcium contributing only to the early phase of post-tetanic potentiation. Supported by NIH Grant NS 15114 and Bell Labs.

486.7

POTENTIATION OF SYNAPTIC TRANSMISSION AT AN IDENTIFIED SYNAPSE IN THE CEREBRAL GANGLION OF APLYSIA. S.M. Fredman. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

The excitatory monosynaptic connection between A and B neurons in the cerebral ganglion of Aplysia exhibits homosynaptic potentiation following high frequency stimulation. Two A and two B neurons were impaled in ganglia bathed in 5x Ca^{2+}, 3x Mg^{2+} SW to suppress polysynaptic pathways. One A neuron was driven at 0.004-0.01 Hz, producing a decrement of its EPSPs in the B neurons. An interposed stimulation of the A neuron with a 2 sec 10-30 Hz train potentiated the EPSPs in both B neurons. The amplitude of the subsequent test EPSPs slowly increased to 150% of their initial (pre-decrement) levels, with the peak increase occurring 5-6 min after the high frequency train. A single 2 sec stimulation produced potentiation lasting longer than 20 min. Stimulating either B neuron or the second A neuron at 30 Hz was without significant effect on the EPSPs evoked by the first A neuron. This suggests a primarily presynaptic mechanism for the potentiation. This work was supported by NIH grant NS20846 and RCMI grant RR03032.

486.8

A NOVEL MOLECULAR MECHANISM FOR PRESYNAPTIC INHIBITION: THE ARACHIDONIC DERIVATIVE HEPOXILIN-A DIRECTLY ACTS ON THE SEROTONIN-SENSITIVE "S" K^+-CHANNEL TO INCREASE ITS OPENING IN APLYSIA SENSORY NEURONS. F. Belardetti, W. Campbell*, P. Chabert*@, J.R. Falck*, C. Mioskowski*@, and M. Rosolowsky*. Depts. Pharmacology and Molecular Genetics, UT Southwestern Med. Ctr., Dallas, TX 75235 and @Lab. De Chimie Bio-Organique, Universite' L. Pasteur, Strasbourg, France 67408.

FMRFamide increases, through the release and transformation of arachidonic acid to 12-hydroperoxy-eicosatetraenoic acid (12-HPETE), the opening (P_O) of the S channel in Aplysia sensory neurons (SNs), but 12-HPETE applied to inside-out SN patches does not increase this P_O. 12-HPETE to act might need to be re-arranged to Hepoxilins A and Hepoxilin B. To test this idea, SNs were incubated with clotrimazole (5 μm), an inhibitor of the re-arrangement. The FMRFamide response was abolished (4/6 cells). Next, 12-HPETE (~160 nM) was applied to inside-out patches in the presence of hematin (1 μM), to catalyze the re-arrangement. Under these conditions, 12-HPETE now produced a sharp and transient, 10-fold increase of the S channel P_O (4/6 patches). In separate experiments without hematin, Hepoxilin A applied to inside-out patches (250-500 nM) produced a similar large increase of P_O (3/5 patches). Vehicle application (N=3) was without effect. Since no GTP or ATP was added, this action on the channel does not involve a G-protein, cGMP, cAMP or a phosphorylation reaction, but appears direct.

486.9

CA^{++}-CHANNELS SUFFICIENT FOR QUANTAL RELEASE FROM CHICK CILIARY GANGLION NEURONS. J.T. Hackett and J.B. Tuttle. Depts of Physiol. and Neurosci., Univ of Virginia School of Medicine, Charlottesvl., Va 22908

Recordings of Ca^{++}-channels involved in quantal release have not been reported. However, synaptic Ca^{++} channel properties must include: low voltage activation, slow or no inactivation, block by Mn^{++} but not dihyrdropyridine, and close proximity to the release site. To record synaptic Ca^{++}-currents, muscle was grown on 1 mm spots of collagen and ciliary ganglion neurons added after myotube formation. This restricts neurite length and the available realm of synaptic interaction. Neurons were whole-cell patch clamped with the pipet containing 150mM CsCl, 10mM EGTA-CsOH, 1 mM $MgCl_2$ and buffered to pH 7.4. The bath had 10 mM $CaCl_2$ and 2 uM tetrodotoxin. Quantal postsynaptic potentials were detected using microeletrodes placed in the muscle cells. In simultaneous recording from nerve and muscle pairs, three conditions were obtained: non-transmitting junctions with only non-synaptic Ca^{++}-channels; low probability release evoked solely by non-synaptic Ca^{++}-channels; one-to-one transmission with synaptic Ca^{++} channels that did not inactivate. These results indicate that intracellular Ca^{++} can trigger release independent of the Ca^{++} channel source. We conclude that synaptic Ca^{++} channels impart strong transmission to these synapses because response characteristics, density and location give a more exclusive linkage to the release mechanism. Supported by NSF BNS 87-08162 and the Am. Heart Assoc. (Va Affiliate).

486.10

SYNAPTIC AND CELLULAR MECHANISMS OF CARBACHOL-INDUCED HIPPOCAMPAL RHYTHMIC SLOW WAVE (THETA). F.W.Y. Tse and B.A. MacVicar, Dept. of Medical Physiology, University of Calgary, Calgary, Alberta, T2N 4N1, Canada.

We recorded intracellularly from CA3 pyramidal neurons in hippocampal slices of 21-28 days old rats. Carbachol (20-60μM) induced 5-10 Hz depolarizations (theta) which occurred synchronously in CA3 neurons and increased in amplitude with membrane hyperpolarization.

Carbachol-induced theta was blocked by atropine, TTX, kyurenic acid (an excitatory amino-acid blocker), or Ca^{++} channel blockers (Cd^{++}, Co^{++} or Mn^{++}); but it was not blocked by bicuculline, 5-7mM external Ca^{++} (which blocks polysynaptic transmission), blockers of NMDA receptors, or removal of the dentate gyrus. Activation of C kinase by a phorbol ester or inhibition of C kinase by preincubation in H-7 neither activated theta nor blocked carbachol-induced theta. However, bath application of inhibitors of intracellular Ca^{++} release (TMB-8 or Ba^{++}) blocked carbachol-induced theta.

We postulate that hippocampal theta involves muscarinic enhancement of a non-NMDA monosynaptic excitation of CA3 neurons by amino-acid releasing interneurons with widespread connections in the CA3 region. Intracellular Ca^{++} mobilization but not C kinase activation is critical in carbachol-induced theta.

486.11

DYNAMIC PROPERTIES OF CHEMICAL TRANSMISSION IN THE CRAB T-FIBER SYNAPSE. J.-W. Lin and R. Llinás. Dept. Physio. & Biophysics., NYU School of Medicine, New York, NY 10016

The crab T-fiber synapse is a tonically transmitting junction with a non-spiking presynaptic element. It is one of the few synapses that allows simultaneous pre- and postsynaptic recordings at the junctional site (Blight and Llinas, Phil. Trans. Roy. Soc., 1980).

Two electrodes were used presynaptically and one postsynaptically. EPSPs were evoked by 50 mV, 3-5 ms presynaptic pulses. A double pulse paradigm revealed a brief facilitation that was followed by a longer lasting depression. The former declined within 150 ms, the latter within a second. Repetitive stimulation with comparable inter-pulse intervals (0.1 to 40Hz) showed mainly depression, i.e. EPSP amplitude was inversely related to stimulus frequency. By contrast, prolonged (300 ms) conditioning pulses (CP), with an amplitude range of 20 to 140 mV, potentiated the test EPSPs up to 6 fold. The potentiation decayed with a time constant of 3.1 sec ($\pm$0.67, n=4). The magnitude and duration of the potentiation increased with larger CPs. As the CP approached the supression level (+80mV), the potentiation was reduced. This result indicates that the potentiation is triggered by the CP-activated calcium influx. Furthermore, the potentiation behaved as a saturable process, since repeated CP (which activated near maximal potentiation if applied individually) had little effect on the magnitude and time course of the potentiation that followed. (Supported by NS07942 and AFOSR85-0368)

486.12

WITHDRAWN

487.1

ONGOING ELECTRICAL ACTIVITY OF SUPERIOR CERVICAL GANGLION CELLS IN MAMMALS OF DIFFERENT SIZE. D. Purves and A. Ivanov, Dept. Anatomy & Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110 and Bogomoletz Inst. of Physiology, Kiev, U.S.S.R.

Studies of the organization of the peripheral sympathetic system have demonstrated systematic differences in the form and innervation of superior cervical ganglion cells among closely related mammals that differ primarily in size (Purves et al., J. Neurosci. 6: 158-163, 1986). Ganglion cells in progressively larger mammals have more elaborate dendritic arbors and receive a greater number of preganglionic inputs. In the present study we asked how these structural and functional differences might affect the ongoing pattern of synaptic activity among individual neurons. Accordingly, we used intracellular recording to monitor ongoing postsynaptic activity from ganglion cells in anesthetized but otherwise intact mice, hamsters, rats, guinea pigs and rabbits.

The proportion of ganglion cells exhibiting postsynaptic activity during a standard period of observation (5 minutes) under urethane anesthesia was least in a small mammal like the mouse (30%), intermediate in animals of intermediate size such as the hamster and rat (48% and 45%, respectively), and greatest in the neurons of the largest animals in the series, the guinea pig (89%) and rabbit (95%). We found, moreover, that the frequency of synaptic activation was also proportional to animal size. The amplitudes of the postsynaptic responses, however, were inversely related to body weight, being largest in the mouse and least in the rabbit.

These differences in activity patterns are presumably related to the functional requirements of animals of different bulk, and raise the interesting question of whether normal activity in other parts of the mammalian nervous system also varies in parallel with animal size.

Supported by USPHS Grants NS 18629 and 11699.

487.2

INFLUENCE OF PERIPHERAL TARGET SIZE ON NORMAL PATTERNS OF SYNAPTIC ACTIVITY OF RAT SUPERIOR CERVICAL GANGLION CELLS. J. T. Voyvodic and A. Ivanov*, Washington Univ. Sch. of Med., St. Louis, Mo. 63110 and Bogomeltz Inst. of Physiology, Kiev, U.S.S.R.

The dendritic complexity of sympathetic ganglion cells can be regulated by changing the relative size of peripheral axonal targets (Voyvodic, Soc. Neur. Abstr., 13:574, 1987). Thus, increasing (or decreasing) the amount of target tissue available to neurons in the rat superior cervical ganglion increases (or decreases) the number and length of ganglion cell dendrites. Here, we have examined whether changes in target size and dendritic geometry are associated with changes in synaptic activation of ganglion cells by preganglionic innervation. The relative target size of neurons innervating the submandibular gland was increased by partially denervating the gland at birth, causing the death of many ganglion cells and an increase in size of the dendritic arbor of the remaining neurons. Patterns of tonic synaptic activity were subsequently measured in adult rats for cells innervating either control or partially denervated submandibular glands.

In control rats, only 11% of the ganglion cells innervating the gland (N=95) exhibited ongoing synaptic activity under urethane anesthesia during a 5 minute period of observation. Experimentally increasing the relative size of the target increased the number of active neurons from 11% to 42% (N=60). Moreover, these cells showed an increased frequency of synaptic activity compared to controls, with superthreshold responses increasing from 0.1 to 0.3/s, and subthreshold EPSPs increasing from 0.1 to 1.5/s.

Therefore, increasing the size of the dendritic arbor by increasing the relative size of the peripheral target is associated with a higher rate of ongoing synaptic activity. We suggest that the increase in ongoing synaptic activity is due to increased preganglionic convergence onto the more complex ganglion cells produced by target enlargement.

This work was supported by NIH grants NS 11699 and NS 18629 to D. Purves.

487.3

ACTIVITY-DEPENDENT DIFFERENCES IN THE NUMBERS OF SYNAPTIC VARICOSITIES AT CRAYFISH MOTOR TERMINALS REVEALED BY LIGHT MICROSCOPY. G.A. Lnenicka and S. LePage*. Dept. Biol. Sci., SUNY, Albany, NY 12222.

Differences in the morphology of phasically and tonically active motor terminals were examined in the closer muscle of the crayfish claw. Lucifer Yellow or HRP was injected into the axon of the phasically active fast closer excitor (FCE), and the tonically active slow closer excitor (SCE). The terminals were subsequently viewed with the light microscope. The SCE terminals had a greater total number of synaptic varicosities, as well as more synaptic varicosities per muscle fiber compared to the FCE terminals. These results are consistent with an earlier serial section EM examination of short lengths of terminal, which demonstrated that the SCE had more synaptic varicosities per terminal length than the FCE. This difference in synaptic varicosities appears to be activity-dependent since the previous study also demonstrated that tonic *in vivo* stimulation of the FCE resulted in an increase in the number of varicosities per terminal length (J. Neurosci. 6: 2252). In this system, the number of synaptic varicosities is correlated with the amount of transmitter released during repetitive stimulation (J. Neurosci. 5: 459). An examination of the time-course of the activity-dependent formation of synaptic varicosities is in progress.

Supported by NSF grant BNS-8720135.

487.4

EFFECTS OF EXPANDED MOTOR UNIT SIZE ON SYNAPTIC EFFICACY AT FROG NEUROMUSCULAR JUNCTIONS. M. Regnier and A.A. Herrera, Dept. Biological Sci., Univ. of Southern California, Los Angeles, CA 90089.

To study mechanisms regulating synaptic efficacy we expanded motor unit sizes in sartorius muscles of adult frogs by crushing the nerve and reducing the number of motor axons reinnervating the muscle.

Synaptic safety margins, measured as the sensitivity of nerve-evoked twitching to lowered $[Ca^{2+}]$, were lower in reinnervated muscles with expanded motor units than in control reinnervated muscles. Low safety margins can be partially explained by low transmitter release from nerve terminals. Intracellular recording revealed that both evoked and spontaneous release of transmitter were lower in muscles with expanded motor units. Nerve terminal lengths did not differ between reinnervated muscles with expanded motor units and control muscles suggesting that transmitter release per unit nerve terminal length was lower in expanded motor units. These results suggest that release and synaptic efficacy are inversely related to experimentally altered peripheral field sizes of motor axons. We are currently investigating whether changes in release or postsynaptic properties from expanded motor units are different at short and long postoperative times. This may provide evidence for mechanisms regulating synaptic effectiveness. Supported by MDA and NSF.

487.5

EFFECTS OF REDUCING MOTOR UNIT SIZE ON THE LEVEL OF POLYNEURONAL INNERVATION IN REINNERVATED ADULT FROG SARTORIUS MUSCLES. M.J. Werle and A.A. Herrera. Biol. Sci. Dept., Univ. of Southern California, Los Angeles, CA 90089-0371.

Motor unit size in reinnervated adult frog (Rana pipiens) sartorius muscles was surgically reduced by excising half the muscle fibers and allowing all sartorius motoneurons to reinnervate the remaining fibers. At varying postoperative times (12 days to 2 years), the muscles were removed, and the level of focal polyneuronal innervation (PI) surveyed using intracellular recording. At all times studied, reinnervated muscles with reduced motor units had a consistently lower level of PI than reinnervated muscles with larger motor units. Since physiological estimates of PI may not be accurate at the earliest stages of reinnervation (6-14 days after nerve crush), we are using histology to estimate PI at these times. In this way, we hope to determine if the consistent difference in the level of PI results from an difference in the rate of synapse elimination at these early times or if the initial level of PI formed in muscles with reduced motor units is smaller.

We have also made histological observations of synaptic structure for all muscles from which intracellular recordings were made. Our findings may offer insight into the relationship between motor unit size and competition for synaptic connections. Funded by NSF and MDA.

487.6

DYNAMIC STRUCTURAL REARRANGEMENTS AT THE GROWING AND ADULT MOUSE NEUROMUSCULAR JUNCTION. R. Hill* & N. Robbins (SPON: R. Lederman) Div. Neuroscience, Case Western Reserve Sch. Med., Cleveland, OH 44106.

In order to establish the extent to which adult nerve terminals engage in remodelling, nerve terminal growth in relation to postsynaptic growth was repeatedly observed in both growing and mature identified mouse neuromuscular junctions (NMJ).

Twenty NMJ's from adult (5-6 mo) and 10 from young (3-4 week) CB6F-1 mouse pectineus muscles were observed. The nerve terminals (NT) were stained in situ with the nontoxic binding fragment of tetanus toxin conjugated to texas red (TTcTR) and the postsynaptic AChR's with bungarotoxin-FITC. The animal was then placed on the microscope stage where a 100x objective, SIT camera and frame grabber boards generate and record the image of the NMJ. Three observations were made of each NMJ: on days 0, 4 and 8 in young mice, and on days 0, 35 and 90 in adults.

The predominant form of growth at immature NMJ's consisted of NT extension followed (within a maximum of 8 days) by extension of the corresponding AChR. NT retractions were infrequent. At the mature NMJ, gross structure did not change appreciably over time (Lichtman, 1987), but many adjustments of morphology did occur. Approximately 50% of the NMJ's exhibited lengthenings of both NT and AChR complexes while approximately 50% showed shortenings. Further, 50% of the junctions showed swinging or "floating" of existing NT-AChR complexes of at least one branch.

Our data indicate that immature NMJ's increase in size and complexity by net outgrowth of NT and subsequently, AChR's. The data argue against the notion that the adult NMJ is structurally inflexible. Rather, the adult structure results from both movement of branches and a dynamic equilibrium between ongoing expansive and retractive events. (Support, NIH AG06641 and AG00105).

487.7

NEUROMUSCULAR JUNCTIONS SHRINK AND EXPAND AS MUSCLE FIBERS CHANGE SIZE: STUDIES IN AN ANDROGEN SENSITIVE MUSCLE. S. M. Breedlove, R. J. Balice-Gordon, and J. W. Lichtman, Dept. Psych., Univ. of Calif., Berkeley and Dept. Anat. and Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

After the first postnatal month, neuromuscular junctions in the mouse sternomastoid are not substantially remodeled, but rather enlarge by simple elongation of existing branches as muscle fibers grow (Balice-Gordon and Lichtman, in prep.). These observations are consistent with the idea that growth of junctions is an essentially passive phenomenon related to growth of postsynaptic muscle fibers. To directly test this, we have used 4-Di-2-ASP staining of nerve terminals and fluorescent bungarotoxin labeling of acetylcholine receptors to repeatedly examine the same junctions in the bulbocavernosus (BC) muscle of sexually mature male mice from 3 to 6 months of age. The BC is involved in copulatory behavior and is sensitive to androgens; castration results in a decrease in muscle mass which can be reversed with testosterone.

As in the sternomastoid muscle, BC junctions enlarge in proportion to muscle fiber growth but are otherwise remarkably stable in their configuration over the several month interval examined. Visual inspection of the BC muscle and surrounding structures 2-4 weeks after castration indicated a decrease in muscle size. Revisualization of junctions showed that many, if not all, nerve terminal branches and receptor sites were still present. However, the size of junctions determined by total area and by branch lengths had declined. Thus, junctions in the BC do not remodel in response to a decrease in muscle fiber size by the retraction of branches, but their overall size is altered in proportion to changing postsynaptic cell size. To reverse this effect, we have administered testosterone after shrinkage and are examining junction morphology as muscle fibers are enlarged.

487.8

PRE- AND POSTSYNAPTIC REMODELING IN ADULT MOUSE MUSCLE INDUCED BY BOTULINUM TOXIN. C.G. Reiness and J.W. Lichtman, Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

Botulinum toxin blocks neuromuscular transmission, which is restored following extensive neuronal sprouting. Using fluorescent stains that mark both nerve terminals and acetycholine receptors (AChRs), we have observed identified botulinum-poisoned synapses over time in adult mice to determine the fate of original synaptic sites during the recovery from intoxication.

The left sternomastoid muscle of adult (20-25g) mice was exposed, stained with rhodaminated alpha-bungarotoxin to label AChRs and with 4-(4-diethylaminostyryl)-N-methylpyridinium iodide to label nerve terminals (Lichtman et al, J. Neurosci. 7:1215, 1987). Both terminals and AChRs were viewed, then botulinum toxin A (Sigma, 0.4 ng in 0.4 ul) was applied to the endplate zone. One to 13 weeks later, muscles were restained as above and reviewed.

Following a 2-3 week period of muscle atrophy, nerve sprouts are detectable over the fibers, and the original endplates appear shrunken but otherwise unchanged. Subsequently the fibers enlarge, and at this time small AChR clusters can often be found in association with the sprouts. The original AChR sites are generally unaltered, but the overlying nerve terminals are spotty and disordered, suggesting that they are retracting. The original endplates then disintegrate, losing nerve terminals and underlying AChRs. Newly forming synapses are frequently detectable nearby on the same fibers. These new sites often have abnormal morphologies and they persist, with minor additions or deletions, throughout the period we have observed.

These results demonstrate that establishment of new synaptic contacts on muscle fibers induces the rapid elimination of the preexisting synaptic sites.

487.9

DISCREPANCIES BETWEEN HISTOLOGICAL AND IN VIVO OBSERVATIONS OF MOTOR NERVE TERMINAL REMODELLING. A.A. Herrera, L.R. Banner*, and N. Nagaya*, Dept. Biological Sci., Univ. of Southern California, Los Angeles, CA 90089.

Until recently, synaptic remodelling at neuromuscular junctions could only be inferred from histological images representing single time points. Repeated observations of junctions in living frogs have now revealed that remodelling commonly occurs even under normal conditions in adults (Soc. Neurosci. Abstr. 13, 1665; 1987).

Analysis of these in vivo images suggests that some of the previous histological results must be re-interpreted: 1) Histology supports the view that terminal sprouts are ephemeral. They either differentiate into mature terminals or retract. We find that terminal sprouts are rather stable, neither growing nor retracting for months. 2) Empty, cholinesterase (ChE)-stained postsynaptic folds are taken as histological evidence of terminal retraction. Indeed, we observe that most retracting branches leave behind ChE-stained folds. However, we also see such folds beyond the distal tips of branches that have grown, suggesting that growth can be followed by partial retraction or that postsynaptic differentiation can precede terminal growth. 3) It was anticipated that remodelling may be more pronounced at polyneuronally innervated junctions, since synapse elimination is prolonged in frogs. Over periods longer than a year, growth is indeed substantial, but multiple inputs are apparently not eliminated. Supported by NIH.

487.10

DIRECT VISUALIZATION OF SYNAPTIC REMODELING IN GASTROCNEMIUS MUSCLES OF LIVING ADULT MICE. D.J. Wigston. Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322.

Dynamic rearrangements of synaptic connections can be observed directly in muscles of living animals, although different muscles may vary considerably in the extent of NMJ remodeling. NMJs in the mouse sternomastoid, a fast-twitch muscle, appear to be remarkably stable (Lichtman et al.,1987; J. Neurosci. 7:1215), whereas about 50% of NMJs in the slow-twitch soleus exhibit some reorganization over 6 months (Wigston, 1987; Soc. Neurosci. Abstr. 13:1007). To explore the possibility that the difference between sternomastoid and soleus in the extent of NMJ remodeling reflects their different physiological type, I examined NMJs in another fast-twitch muscle, lateral gastrocnemius (LG). I labeled acetylcholine receptors at LG NMJs in anesthetized animals with rhodamineα-Bungarotoxin and presynaptic terminals with the fluorescent dye 4-Di-2-ASP (Lichtman et al.,1987). Using low levels of illumination I obtained images of superficial NMJs with a SIT camera and digital image processor. Three months later I restained and examined the same NMJs. Remodeling, in the form of additions and deletions of branches, was evident at some NMJs but less frequently than I observed in soleus muscles monitored over the same time. The extent of remodeling at mammalian NMJs might thus depend on the amount or frequency of their activation.

487.11

NEURON/GLIAL RELATIONSHIPS OBSERVED IN LIVING MICE. S.L. Pomeroy and D. Purves, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The relationship of salivary duct ganglion neurons and their associated glia was assessed by videomicroscopy in anesthetized but otherwise intact mice. Adult males (CF1 strain; 25-30g) were placed on the stage of a modified microscope and the salivary duct ganglia were exposed as previously described (Purves et.al., Science 238: 1122-1126, 1987). Video images were obtained using a 100X water immersion objective.

The arrangement of particular neurons and the 1-3 glia associated with them was assessed by filling single glial cells with 5(6)-carboxyfluorescein after impalement with a microelectrode. In all cases (n=75), the entire neuron was enveloped by a glial sheath stained with fluorescent dye. This result indicates that each glial cell is associated with a single neuron, and that when more than one glial cell is associated with a neuron it is strongly dye coupled to the other glia investing that cell. The stability of the neuron/glia relationship was determined by repeated examination of cells over intervals up to 130 days. While no changes were noted after 6 hours, an increasing percentage of neurons showed change in number and/or location of associated glial nuclei over progressively longer intervals. After 110-130 days, 80% of the neurons showed change in these parameters.

Finally, examination of electron micrographs of 192 salivary duct neurons revealed that preganglionic nerve terminals are much more prevalent in the vicinity of glial nuclei than elsewhere on the neuronal surface. This association of nerve terminals and glial nuclei, which in turn change in number and/or location over time, is in agreement with the recent observation that preganglionic terminals are gradually remodelled. This association also suggests that glia play a part in ongoing synaptic rearrangement. Supported by USPHS grants NS 18629, 11699 and 07027.

487.12

DYNAMIC RELATIONSHIP BETWEEN SYNAPTIC EXTRACELLULAR MATRIX AND MOTOR NERVE TERMINALS IN LIVING FROGS. L. Chen* & C.-P. Ko (SPON: J.H. Caldwell). Dept. Biol. Sci., Univ. Southern California, Los Angeles, CA 90089.

Rhodamine-labelled peanut agglutinin (PNA) specifically stains the synaptic extracellular matrix at living frog neuromuscular junctions (NMJs). To examine the remodeling of synaptic matrix and its dynamic relationship with nerve terminals in identified NMJs of living adult frogs (R. pipiens), sartorius muscles were stained with 4-di-2-Asp, a fluorescent dye for motor nerve terminals (10uM, 3 min), followed by PNA (50ug/ml, 30 min). NMJs were observed in anesthetized frogs in situ with fluorescence microscopy and videotaped with a SIT camera. After about 2 mos., the same muscles were restained and identified NMJs reexamined. Preliminary results show changes in synaptic matrix and/or nerve terminals in more than half of NMJs observed. Some NMJs increase or decrease equally in both matrix and terminal length. In others, either the terminal extends while the matrix retracts, or the terminal retracts while the matrix is unchanged. In one junction viewed 3 times, an extension (30 um) of both matrix and terminal was observed after 2.5 months. A third imaging done 6 weeks later revealed a decrease (24 um) in the terminal but a slight increase in the matrix length.

Results suggest that remodeling occurs in nerve terminals and synaptic matrix in adult frog NMJs. Also, changes in nerve terminal length and synaptic matrix do not necessarily correspond.

488.1

PRIMATE SELECTIVE BRAIN RETENTION OF TC99M-ECD: A NEW CEREBRAL PERFUSION AGENT. R. C. Walovitch, M. Watson*, M. Ganey*, and S. J. Williams*. E.I. DuPont de Nemours & Co., (Inc.), N. Billerica, MA 01862.

Tc99m-ethyl cysteinate dimer (ECD) is a neutral, lipophilic complex which rapidly crosses the blood brain barrier. It is presently being evaluated clinically as a marker of regional cerebral perfusion. Multispecies pharmacological evaluation of Tc99m-ECD show the compound to be efficacious as a brain perfusion agent only in primates. The brain pharmacokinetics of Tc99m-ECD are similar in humans and in non-human primates. Rapid brain uptake (initial 5% injected dose) was observed in both species, as was prolonged retention in a cerebral perfusion pattern (Neurology 38:363,1988). Results in humans and nonhuman primates suggest that the brain retention and extensive renal clearance of Tc99m-ECD are due to its rapid metabolism via ester hydrolysis. Subcellular distribution studies in monkey brain one hour post Tc99m-ECD administration demonstrate that more than 70% of the activity is localized in the cytosolic fraction and is primarily in the form of single polar metabolite. When this metabolite was injected into a monkey it failed to cross the blood brain barrier. These results support the hypothesis that Tc99m-ECD is metabolized rapidly in the brain of primates by a specific enzymatic pathway to a polar complex which is trapped.

488.2

CORTICAL CEREBRAL BLOOD FLOW (CBF) IS INCREASED BY ELECTRICAL STIMULATION OF THE BASAL FOREBRAIN (BF): MODULATION BY CHOLINERGIC MECHANISMS. S.P. Arnerić. Dept. of Pharmacol., Southern IL Univ. Sch. Med., Springfield, IL

We sought to determine: 1) Does electrical stimulation of the BF alter cortical CBF? 2) If so, are cholinergic receptors involved? Rats were anesthetized (chloralose), paralyzed, artificially ventilated and arterial blood gasses controlled. A craniotomy was performed over the parietal cortex and microvascular perfusion, an index of CBF, measured continuously with laser-doppler flowmetry. Electrical stimulation of the BF with a bipolar concentric electrode elicited remarkable increases in cortical CBF (up to 250% of resting CBF) that were frequency (2.5-100 Hz) and intensity (25-150 μA) dependent; but independent of heart rate or arterial pressure (N=7). A nicotinic antagonist, mecamylamine (4 mg/kg, i.v., 20 min prior) significantly attenuated by 52±8% the BF-elicited increase ($p<0.05$; N=4). In contrast, the response was enhanced by 40±15% ($p<0.05$; N=3) with a cholinesterase inhibitor, physostigmine (50 μg/kg, i.v.). Surprisingly, a muscarinic antagonist, atropine (1 mg/kg, i.v.) also enhanced the effect similarly. CONCLUSIONS: 1) Cortical CBF is profoundly increased by electrical stimulation of neurons originating in or passing through the BF; 2) cholinergic neurons, possibly those in the BF, participate in this response; and 3) nicotinic receptors facilitate, while muscarinic receptors inhibit this dilator pathway (Supported by American Health Assistance Foundation).

488.3

STATE-DEPENDENT DIFFERENCES IN CEREBRAL BLOOD FLOW DURING CORTICAL SPREADING DEPRESSION IN RAT. R. B. Duckrow. Department of Medicine, Division of Neurology, The Pennsylvania State University, Hershey, PA 17033.

Cortical spreading depression (SD) is a reversible phenomenon that may occur during complicated and classic migraine headache. In anesthetized rats regional cerebral blood flow (rCBF) increases dramatically as SD advances through the neocortex. However, hyperemia has not been measured in the cortex of awake humans during migraine. The hypothesis that these flow differences are state-dependent was tested by measuring rCBF in awake and anesthetized rats during SD. Using pentobarbital anesthesia a bipolar electrode was placed on the lateral convexity of the left hemisphere and secured with dental acrylic. Two days later rats were prepared under halothane/nitrous oxide anesthesia, restrained with a plaster hip-cast and allowed a one hour recovery period. SD was induced by a 5 mA direct current lasting 5 seconds and confirmed by recording electrocortical activity. Two minutes later rCBF was measured using 14C-iodoantipyrine in regions isolated by gross dissection. In rats reanesthetized with pentobarbital, rCBF in the hemisphere with SD increased by 40%. In awake rats rCBF decreased by 30% in the SD hemisphere and increased by 50% in the contralateral hemisphere. These state-dependent differences in rCBF during SD are consistent with the presence of an intrinsic neural system regulating blood flow in the brain. (Supported by PHS NS24109 and an AHA Established Investigator Award)

488.4

BLOOD FLOW IN NEURAL GRAFTS. S.C. Jones, E. Korfali*, W.D. Knowles, S.M. Chou*, J.R. Little*. Cerebrovascular Research Lab., Cleveland Clinic Foundation, Cleveland, OH 44195.

The extent and amount of blood flow and tissue perfusion in neural grafts is important in the evaluation of neural grafts for the treatment of Parkinson's and Alzheimer's disease and for cerebral ischemia.

Nine Sprague Dawley rats weighing 180-200 g were prepared with a 2x3 mm cavity for transplantation. Two weeks later, embryonic septal tissue from 15-16 day old embryos was placed in five of these animals. Four were not grafted. All surgical procedures were performed under barbiturate anesthesia (Nembutal 40-50 mg/kg, i.p.). Five months after transplantation (body weight ± SEM = 480 ± 8 g), cerebral (and graft) blood flow (CBF) was determined using quantitative autoradiography (Sakurada et al., Am. J. Physiol. 234:H59, 1978) under physiologically controlled and stable conditions of mean arterial blood pressure (83 ± 7 mmHg), $PaCO_2$ (34.8 ± 0.7 mmHg), PaO_2 (170 ± 6 mmHg), and pH (7.422 ± 0.012). Grafts were differentiated from host tissue in Nissl stained sections.

CBF ± SEM for parietal cortex of all animals were 66 ± 5 ml/min/100 g, normal for pentobarbital anesthetized rats. Graft and contralateral-to-graft flows were 37 ± 3 and 51 ± 6 ($p < 0.1$), compared to contralateral-to-graft site in the non-graft animals of 64 ± 11.

Grafted animals had normal CBFs in adjacent-to-graft cortex, whereas grafted tissue had blood flows that were lower than normal brain except for one animal. Grafted tissue appears to develop perfusion that provides lower blood flow than normal brain.

488.5

LOCAL SYMPATHETIC CONTROL OF THE CEREBRAL CIRCULATION: AN AUTORADIOGRAPHIC STUDY. U.I. Tuor. Division of Biomedical Research, Hospital for Sick Children, 555 University Avenue, Toronto, Canada M5G 1X8

The present study examined the effect of sympathetic stimulation on the local cerebral circulation. Changes in local cerebral blood flow were measured in anesthetized rats during unilateral stimulation of the superior cervical ganglion using ^{14}C-iodoantipyrine autoradiography. Stimulation parameters were chosen on the basis of preliminary studies in 10 rats, which demonstrated that stimulation of the ganglion at 15 Hz, 3 msec and 10 V produced a 1-2 mm Hg reduction in cerebral venous (sagittal sinus) pressure indicating an overall reduction in cerebral blood volume and/or flow. In sham control animals (n=4), changes in local cerebral blood flow with respect to the contralateral hemisphere were less than 2% in all regions examined. During unilateral stimulation of the sympathetic ganglion (n=5), blood flow in temporal muscle was reduced by 70±12% (mean±SEM) whereas there were less marked reductions in local cerebral blood flow. For example, blood flow in the cerebellar cortex was similar in both hemispheres. Blood flow in the parietal cortex and the caudate nucleus were reduced ipsilateral to the stimulation by 9.3±2.8% and by 11.0±5.4%, respectively. Reductions in flow were unevenly distributed within these regions possibly corresponding to a differential regional density of sympathetic innervation of the cerebrovasculature. Further experiments and analysis are required to map in detail the influence of sympathetic stimulation on the cerebral circulation.

488.6

CHANGES IN REGIONAL CEREBRAL BLOOD FLOW AND SUCROSE SPACE AFTER 3-4 WEEKS OF HYPOBARIC HYPOXIA(0.5ATM). J.C. LaManna, K.A. McCracken*, and K.P. Strohl*. Depts. Neurology, Medicine and Physiol./Biophys., Case Western Reserve University Cleveland, OH 44106, U.S.A.

Chronic hypoxia results in increased ventilatory rate and hematocrit, with decreased PaCO2, which have important consequences for cerebral vascular function. In this study, adult Wistar rats were kept in a hypobaric chamber for 3-4 weeks at 0.5ATM. Rats were studied either 4 or 24 hours after removal from the chamber. Regional cerebral blood flow (BF) and sucrose space (SS) were measured by the dual-label single pass indicator fractionation method using 14C-butanol and 3H-sucrose in cerebral and cerebellar cortex, hippocampus, and striatum. Rats were anesthetized with chloral hydrate (400mg/kg, i.p.) for placement of arterial and venous cannulae, and allowed to recover. 4 hrs after being returned to normobaric normoxia, arterial CO2 was still decreased, arterial pH was acidic and hematocrit was elevated. BF in all regions was about 3 times control. SS in these rats was depressed but, when considered with the rise in hematocrit, indicated that regional blood volume was probably not altered. BF and SS at 24 hrs were the same as control rats. Thus, hypoperfusion due to hyperventilation does not occur in chronic hypoxia. The cerebrovascular effects of chronic hypoxia persist for some hours after return to normoxia. We conclude that there must be long-term changes in the control mechanism of the cerebral vasculature during acclimatization to hypoxia.

488.7

REGIONAL VARIATION IN BRAIN GLYCOGEN CONCENTRATION AND CATABOLISM IN SUCKLING RABBITS, R.S.Rust* (SPON: M. Noetzel) Depts. of Neurol. and Pediatr., Washington Univ. Sch. of Med., St. Louis, MO 63110.

Histological studies have identified brainstem and deep cortical radial glial cells as important loci for deposition of glycogen during early neural development, but regional variation in concentration and utilization of this glycogen has not been well characterized. Nine five day old rabbit pups were killed either by direct immersion in liquid nitrogen or by rapid freezing of heads at 30 or 120 second intervals after decapitation in order to assess changes in glucose, glycogen, G6P, ATP, and lactate in ten brain regions, measured by standard enzymatic methods after appropriate extraction of excised tissues. Glycogen concentration in parietal cortex (2.0±0.41mM/kg) was similar to that found throughout mature rabbit brain. Significantly greater concentrations ($p \leq 0.001$) were found in subiculum (3.89±0.58), midbrain (5.95±0.85), cerebellum (7.65±1.12), medulla (9.86±0.67) and cervical spinal cord (12.71±1.24). Total ischemia resulted in 25-44% reduction in glycogen concentration in midbrain, pons, medulla, and cord, but little if any change in cortical or cerebellar regions. Regional rates of glucose, PCr, and ATP and for accumulation of lactate roughly paralleled glycogen utilization. These results suggest that glycogen enrichment in certain functionally critical areas of developing brain may provide a rapidly mobilizable energy reserve during total ischemia.

488.8

TOTAL OR PARTIAL BRAIN ISCHEMIA: A NEW RAT MODEL. J.C. de la Torre, T. Fortin* and J. Thakar. Univ. of Ottawa Health Sciences, Ottawa, Ont.

Experimental stroke models using the rat have been developed to produce partial or total arrest of blood flow to brain but no model offers both options. The four-vessel total ischemia model and several modifications of it, hinges on the fact that rats remain symptomless if fewer than 3 vessels to the brain are occluded (e.g., both vertebral arteries). This model was difficult to reproduce because vertebral artery electrocautery through the alar foramina often resulted in brain tissue damage or undesirable bleeding despite direct visualization of the vessel. The present model was developed because the rat offers many advantages over other animal models. Rat is anesthetized with diazepam-somnotol and xylocaine is sprayed orally to reduce gag reflex. Rat is intubated with a PE 190 catheter and ventilated mechanically. After a mid-thoracic incision, a hemostat is used to clamp the left costo-sternal attachments from just above the xiphoid process to just below the first rib. An electrocoagulating incision is made between sternum and hemostat to expose the heart. The aortic arch great vessels are identified and the left and right subclavian arteries are occluded with microvascular clips. The chest wall is closed with sutures and 2 ligatures are looped around both common carotid arteries then threaded externally via catheters. After rat fully recovers, one or both carotid ligatures are pulled and released for varying periods of time. This procedure results in either partial or total cerebral ischemia compunded by reperfusion damage. Pilot studies show that this 3 or 4 vessel occlusion model can be easily and reliably set-up to study morphologic, physiologic and chemical brain pathology under moderate or severe circulatory parameters.

Supported by the Ontario Heart Foundation.

488.9

Recovery of Impaired Endothelium - Dependent Relaxation Following Concussive Brain Injury. M.D. Ellison*, D.E. Erb*, J.T. Povlishock (Spon. J. Astruc) Dept. of Anat., VA Commonwealth Univ., Richmond, VA 23298

Endothelium dependent relaxing factors (EDRF) are agents of undetermined chemical identity, produced by normal endothelium, which effect vasodilation in response to certain agonists. Recently, it has been shown that feline pial microvessels produce an EDRF when exposed to topically-applied Ach and that such a response is abolished immediately following experimental fluid percussion brain injury. Whether the post-traumatic impairment of endothelial dependent relaxation (EDR) is reversible, over time, has not been determined and was examined in the present study. Aseptically, anesthetized cats were each equipped with a cranial window and subjected to a moderate level of brain injury. Pial arteriolar EDR following Ach exposure was assessed prior to injury and at 30 minutes, 4, 8 and 12 hours post-trauma. At each assessment, vascular diameters were measured before and after Ach application. At 30 minutes post-injury, normal Ach-induced EDR was converted to vasoconstriction, most notably in small caliber vessels (<100μm). At 4 hours, recovery of EDR was observed in a number of vessels, and by 12 hours the majority of vessels exhibited a normal response, although some remained dysfunctional. Small arterioles recovered sooner than large ones. The findings suggest that, in the absence of secondary insults, mechanisms underlying early posttraumatic loss of cerebral EDR do not persist and do not, for the most part induce irreversible changes which prevent recovery of EDR. Those vessels, which fail to demonstrate EDR at 12 hours post-trauma, however, may require more time for recovery or may be irreversibly damaged. Supported by NIH Grant NS 12587

INTERACTIONS BETWEEN NEUROTRANSMITTERS III

489.1

ANTIPSYCHOTIC DRUGS INCREASE NEUROTENSIN CONCENTRATIONS AFTER DESTRUCTION OF DOPAMINE NEURONS BY 6-HYDROXYDOPAMINE. C. Bissette, K. Dole, M. Johnson*, D. Knight* and C.B. Nemeroff. Depts. of Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.

The chronic administration of clinically effective antipsychotic drugs increases the concentration of neurotensin (NT) in the anterior caudate nucleus and nucleus accumbens of rat brain. Because neuroleptic drugs block the dopamine (DA) receptors that are found in these regions containing terminal projections of mesencephalic DA neurons, we sought to determine whether the presynaptic DA neurons were essential for this neuroleptic-induced increase in neurotensin concentration. Adult, male, Sprague-Dawley rats were pretreated with desmethylimipramine (30 mg/kg, IP) one hour before intracisternal injection of either 6-hydroxydopamine (6-OHDA, 200 μg) or 0.1% ascorbic acid vehicle. This procedure was repeated after seven days and results in a greater than 90% depletion of DA in the caudate nucleus. One week after the last 6-OHDA injection, some animals from both groups were injected daily with either haloperidol (1 mg/kg, IP) or 0.3% tartaric acid vehicle for three weeks. Rats were decapitated and the following brain regions dissected for radioimmunoassay of NT: frontal cortex, olfactory tubercles, nucleus accumbens, anterior caudate, septum, pre-optic/diagonal band, hypothalamus, amygdala, hippocampus, ventral tegmental area/substantia nigra and cerebellum.

Both the 6-OHDA treated group and vehicle controls receiving haloperidol had significant ($p<0.001$, ANOVA) increases in NT concentrations in the nucleus accumbens and anterior caudate nucleus compared to the groups that did not receive haloperidol. Thus, while the postsynaptic dopamine receptor is needed for the neuroleptic-induced increase in NT concentrations in these regions, the dopamine neuron is not primarily involved. Supported by NIMH MH-35415 and a grant from the Schizophrenia Research Foundation.

489.2

ASYMMETRICAL DISTRIBUTION OF NEUROTENSIN (NT)-IMMUNOREACTIVITY FOLLOWING UNILATERAL INJECTION OF 6-HYDROXYDOPAMINE IN RAT VENTRAL TEGMENTAL AREA (VTA). S.N. Johnson* and D.S. Zahm. (SPON:R.Walsh) Dept. of Anat. and Neurobiol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

It has been shown that IP administration of haloperidol (HAL) is followed within 24 hours by increases in the numbers of NT-IR neurons selectively in the ventral striatum (Eggerman and Zahm, Neuropeptides 11(3):125-32, '88). We now report a similar but more dramatic response following unilateral 6-hydroxydopamine (6OHDA) injections in the VTA. One week following such injections, NT-IR neurons were 10- to 50-fold more numerous in striatal districts on the lesioned as compared to unlesioned sides of the brains. In contrast to conditions following DA receptor blockade, the distribution of NT-IR neurons extended to include dorsal striatal territories after 6OHDA lesions. The extreme dorsolateral quadrant, however, always remained devoid of NT-IR neurons. The moderate expansion of the NT-IR pallidal districts observed after HAL treatment was more pronounced after 6OHDA. The NT-IR territory of the globus pallidus on the lesioned side was 1.5-2.5 times larger that its counterpart on the unlesioned side. The dorsolateral ventral pallidal district that normally exhibits sparse NT-IR and gives rise to projections terminating in the substantia nigra and subthalamic nucleus (see Zahm and Johnson, this volume) displayed intense NT-IR following the chemical lesion. Support: NIH NS-23805 and the American Parkinson Disease Association.

489.3

NEUROTENSIN INNERVATION OF DOPAMINE NEURONS IN RAT VENTRAL TEGMENTUM. J. Woulfe* and A. Beaudet. Montreal Neurological Institute, Montreal, Canada, H3A 2B4.

Several lines of evidence advocate a functional interaction between neurotensin (NT) and dopamine (DA) neurons in the brain. Among these is the selective association of NT high affinity binding sites with DA neurons in the substantia nigra, pars compacta (SNC) and ventral tegmental area (VTA) of the rat. To further examine the morphological substrate underlying NT-DA interactions in the ventral midbrain, we have employed a light and electron microscopic sequential double antigen localization technique for simultaneous visualization of NT (using 3'3 diaminobenzidine as the chromogen) and the dopamine biosynthetic enzyme tyrosine hydroxylase (TH, using benzidine dihydrochloride as the chromogen). At the light microscopic level, NT-immunoreactive terminals were densely distributed throughout the VTA and SNC in close proximity to TH-immunoreactive somata and dendrites. On electron microscopic examination, direct axosomatic and axodendritic synaptic connections were identified between NT-immunoreactive axon terminals and TH-immunoreactive perikarya and dendrites. The present results provide morphological evidence for a direct NT innervation of DA neurons in the VTA and SNC. This is consistent with a neural influence imparted by NT on the activity of DA systems arising from these areas. Supported by MRC.

489.4

ENHANCEMENT OF DOPAMINE METABOLITES IN THE NUCLEUS ACCUMBENS USING MICRODIALYSIS PERFUSION AFTER INJECTION OF NEUROTENSIN AND CHOLECYSTOKININ INTO THE VENTRAL TEGMENTAL AREA. Ph. De Witte(1), J. Crawley(2) and I. Mefford(3). (1) Lab. Psychobiology, Univ. Louvain, B-1348 Louvain-la-Neuve, Belgium; (2) Lab. Behav. Neuroscience and (3) Lab. Biochemistry, National Institutes of Mental Health, Bethesda, MA 20817, USA.

High densities of neurotensin-cholecystikinin-and dopamine-containing perikarya were found in the ventral tegmental area (VTA). Coexistence between these peptides and dopamine in the fibers of the mesolimbic pathways originating from the VTA and projecting to the nucleus accumbens suggested a neuromodulation on the dopaminergic transmission by these peptides. The aim of the present study was to estimate the level of concentration of the extracellular metabolites of dopamine, i.e. DOPAC and HVA, and serotonine, i.e. 5HIAA, in the nucleus accumbens after microinjection of neurotensin of cholecystokinin (10 pmodes and 10 nmoles) into the VTA. The concentration of these metabolites was estimated using high pressure liquid chromatography with electrochemical detection. Our results show that the injection of distilled water and the low dosage (10 pmoles) of neurotensin or cholecystokinin remain without effect. The injection of saline gave rise to transient increase in DOPAC while 10 nmoles of both peptides induced a larger and longer increase in the extracellular DOPAC and HVA while 5-HIAA never changed from the baseline level, in the nucleus accumbens after their VTA microinjections.

489.5

CHOLECYSTOKININ REVERSES A NEUROTENSIN-INDUCED INCREASE IN DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS: AN IN VIVO ANALYSIS. A.G. Phillips[1], A. Lesage[1], C.D. Blaha[1,2], and H.C. Fibiger[2], Depts. of Psychology[1] and Psychiatry[2], Univ. of British Columbia, Vancouver, BC, Canada, V6T 1Y7.

The neuropeptides cholecystokinin (CCK8-S), neurotensin (NT) and the catecholamine dopamine (DA) have been shown to be colocalized in mesotelencephalic neurons. Using in vivo chronoamperometry (60s pulse rate), we have shown previously that intracerebroventricular (icv) injection of NT (0.1-10 ug/10 ul) induced an immediate dose-dependant increase in DA release in the nucleus accumbens. In contrast icv administration of CCK8-S (5-100 ng) inhibited DA release in this region of the rat brain. In vivo microdialysis (1 ul/min;10-12 min dialysates) in combination with microbore HPLC-EC permitted the measurement of DA and its major metabolites in the nucleus accumbens. These analyses confirmed a significant increase in extracellular DA levels after icv administration of comparable doses of NT. Chronoamperometric studies also revealed an immediate reversal of the NT-induced increase in the DA signal when CCK8-S was administered 60 min after NT. Parallel studies with microdialysis are presently ongoing to confirm the antagonistic effect of CCK8-S on NT modulation of DA neurotransmission.

489.6

OPIOID INHIBITION OF AMINO ACID ELICITED CATECHOLAMINE RELEASE FROM HIPPOCAMPAL AND STRIATAL SLICES OF RAT BRAIN. L.L. Werling, P.N. McMahon* and B.M. Cox. Dept. of Pharm., Uniformed Services University, Bethesda, MD 20814-4799.

We have previously reported that the potassium (K^+)-stimulated release of [^{3}H]NE from slices of rat hippocampus can be inhibited in a dose-dependent manner by opioid agonists which act through the mu receptor, but not by those acting through delta or kappa receptors. In contrast, K^+-stimulated release of [^{3}H]DA from striatal slices can be inhibited by kappa opioid agonists, but not by mu or delta agonists. We now report that release of these catecholamines can be stimulated by glutamate and N-methyl-D-aspartate (NMDA) in a dose-dependent manner. Quisqualate and kainate were much less effective in stimulating release. Release stimulated by 100 μM NMDA could be blocked by the antagonist 2-amino-5-phosphono-valeric acid (APV, 50 μM) or by the inclusion of Mg^{+2} in the incubation medium. Consistent with the pharmacological profile for inhibition of K^+-stimulated release, the mu-opioid agonist Tyr-D-Ala-Gly-NMePhe-Gly-ol inhibited NMDA-stimulated [^{3}H]NE release from hippocampal slices, while the kappa agonist U-50,488H inhibited NMDA-stimulated [^{3}H]DA release from striatal slices over concentration ranges equivalent to those required for inhibition of K^+-stimulated release. Taken together, these data suggest that amino acids which interact with NMDA receptors, and opioids, acting through mu or kappa receptors, may participate in local regulation of catecholaminergic pathways in rat brain.

489.7

CHRONIC STRESS IN MICE ALTERS DOPAMINERGIC FUNCTIONING IN MESOLIMBIC SYSTEM THROUGH ENDOGENOUS OPIOIDS. S. Puglisi-Allegra, S. Cabib*and A. Oliverio* Istituto di Psicobiologia e Psicofarmacologia, C.N.R., via Reno 1, 00198 Roma, Italy

Repeated exposure (10 days) to immobilization (2 h) produces a change in sensitivity to the effects of apomorphine on stereotypic climbing behavior in mice tested 24 h after the last stressful experience. This effect was prevented when naltrexone was administered before each stress session, indicating that alteration of dopamine (DA) functioning induced by chronic stress is mediated through endogenous opioid system.

Biochemical analysis of DA systems response to 2 h immobilization revealed an increase of DOPAC/DA and HVA/DA ratios and a decrease of 3MT/DA ratio in the nucleus accumbens (NAS) and the caudatus-putamen (CP). An increase of HVA/DA ratio was also found in the frontal cortex (FC). Naltrexone administered before immobilization antagonized the decrease of 3MT/DA ratio in NAS but not in CP. Moreover, apomorphine administered in chronically stressed animals was shown to produce stronger effects on DA metabolites in NAS while no significant difference was observed in CP and FC. These results show that immobilization stress inhibits DA release in NAS through endogenous opioids. Moreover, they suggest that repeated stressful experiences alter DA receptor sensitivity in mesolimbic system.

489.8

NEUROTRANSMITTER INTERACTIONS IN THE DORSAL STRIATUM OF THE RAT STUDIED BY IN VIVO MICRODIALYSIS. G.Damsma, H.C.Fibiger, A.S.Horn and B.H.C.Westerink. (SPON: S.R.Vincent). Div. of Neurological Sciences, Univ. of Britisch Columbia, Vancouver, B.C., Canada, V6T 1W5 and Dept. Medicinal Chemistry, Univ. of Groningen, A.Deusinglaan 2, 9713 AW Groningen, The Netherlands.

Neuronal interactions in the striatum of freely moving rats have been studied using a microdialysis sampling technique. On-line connection of the dialysis outlet to sensitive HPLC equipment enabled us to determine directly the dialysate concentrations of dopamine and acetylcholine which are most likely representing the release of these neurotransmitters (Westerink, B.H.C., et al., Life Sci., 41:1763, 1987).

Rats received various drugs, e.g. dopaminergic agonists, neuroleptics, cholinomimetics, anticholinergics, and anesthetics. The results indicate that there is neither a muscarinic modulation of the dopamine release nor a dopaminergic modulation of cholinergic release. These data challenge the well-established hypothesis of a mutual interaction between dopaminergic nigro-striatal terminals and cholinergic striatal interneurons.

489.9

CENTRAL CHOLINERGIC INNERVATION OF THE DOPAMINERGIC AND SEROTONERGIC SYSTEMS. B.K. Hartman, P.L. Faris*, S.J. Kalmbach*1, C. Cozzari*2, A. Berod*3. Dept. Psychiatry, Univ. of Minn, Mpls. MN 55455, Wash. U. Sch. Med. St. Louis MO, Inst. Biol. Cell., Rome, It., Inserm U-171, Lyon, Fr.

Double label immunohistochemistry was used for the simultaneous visualization of choline acetyltransferase (CAT) and tyrosine hydroxylase (TH) or serotonin (5-HT) to investigate the interactions between the pontomesencephalic cholinergic group of neurons (PMCG) and the dopamine and 5HT systems in rat brain. First, CAT was localized using the PAP technique with nickel-cobalt enhanced DAB. Sections were then processed for either TH or 5-HT localization using PAP with alpha-napthol as substrate and differentiated with the dye pyronin-B. The jet-black CAT positive cells and processes contrasted well with the bright magenta staining of the TH or 5-HT positive structures, making evaluation of interactions feasible at the light microscopic level.

The ventral-rostral part of the PMCG extended to the caudal limit of the dopaminergic neurons of the substantia nigra. CAT-positive axon fibers were observed to enter the nigra where they formed numerous bouton-like contacts with TH-positive dendrites and soma. Interactions were restricted to the caudal half of the nigra and the ventral tegmental dopamine neurons. The dorsal part of the PMCG was observed to send axon fibers medially and rostrally into the dorsal raphe where a small number of interactions with 5-HT-positive neurons occured. CAT-positive axons then turned ventrally to enter the midline raphe, again making contact.

On the basis of these results and previously reported interactions between this cholinergic group and the noradrenergic neurons, it is hypothesized that an important function of the PMCG is to coordinate the functions of the three biogenic amines. Supported by NS-12311 (BKH), RSDA MH-00595 (PLF).

489.10

EVIDENCE FOR A FUNCTIONAL INTERACTION BETWEEN THE SEROTONIN (5-HT) REUPTAKE PROCESS AND α_2-ADRENERGIC RECEPTORS LOCATED ON 5-HT TERMINALS IN THE RAT HYPOTHALAMUS. P. Blier, A.M. Galzin* and S.Z. Langer. Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France

The efficacy of presynaptic α_2 receptor agonists to inhibit the electrically-evoked release of [^{3}H]-monoamines from preloaded brain slices was shown to be attenuated in the presence of reuptake blockade for the 5-HT and the noradrenaline (NA) systems. There is controversy, however, as to the involvement of a functional link between the presynaptic receptors and the reuptake carriers or of a competition between the exogenous agonist and the neurotransmitter for the receptor sites. In order to verify the concept that such a functional interaction could exist, we undertook to study the α_2-adrenergic-mediated inhibition of electrically-evoked [^{3}H]-5-HT release from preloaded slices of the rat hypothalamus, a model in which endogenous NA does not reach the NA heteroreceptors located on 5-HT terminals. Two periods of electrical stimulation were applied 60 min (S_1) and 104 min (S_2) after the onset of superfusion. Drugs were added 20 min before S_1 or S_2. The NA reuptake blocker desipramine (0.3 µM) did not alter the electrically-evoked release of [^{3}H]-5-HT or the inhibition produced by UK 14.304 (0.001-10 µM), an α_2-adrenergic agonist. The 5-HT reuptake blockers citalopram (0.01-1 µM) and paroxetine (1 µM), which by themselves did not modify [^{3}H]-5-HT release, decreased the inhibition of [^{3}H]-5-HT release produced by UK 14.304. The effect of exogenous NA (0.1-1 µM) on [^{3}H]-5-HT release was also attenuated in the presence of citalopram. In contrast, citalopram modified neither the electrically-evoked release of [^{3}H]-NA nor the UK 14.304-mediated inhibition of [^{3}H]-NA release. The interaction was also present in slices obtained from rats with depleted endogenous stores of 5-HT. Activation of either protein kinase C or of adenylate cyclase together with phosphodiesterase inhibition, which attenuate the UK 14.304-inhibition of [^{3}H]-5-HT release, did not hamper the interaction between UK 14.304 and citalopram. In conclusion, the presynaptic NA heteroreceptor and the 5-HT reuptake carrier appear to be functionally linked. This interaction cannot be attributed to an increased synaptic availability of either NA or 5-HT or to an activation of either the phosphatidylinositol cycle or the adenylate cyclase system.

489.11

DEVELOPMENT OF DEPOLARIZATION INDUCED GLUTAMATE RELEASE AND ITS MODULATION BY SEROTONIN IN CORTICAL NEURONS. L. Sikich and R. D. Todd. (SPON: E. Robins) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

Glutamate has been postulated to play roles in both normal developmental processes such as synaptogenesis and in pathological processes such as perinatal neuronal death from asphyxia. However, the developmental time course of glutamate release and of its modulation by other neurotransmitters has not been well characterized. In adults, glutamate release is elicited by depolarization and is inhibited by serotonin (5HT). In the cerebellum, depolarization induced glutamate release can not be demonstrated until the granule cells begin to mature at PND 14-20. Neurons in the cortex mature much earlier and might develop glutamate release during embryonic life. However, this has not been examined. Likewise, the development of sensitivity to 5HT has not been examined. This work addresses both questions in a dissociated cell culture system.

Cortices from E13 to E22.5 fetal rats were dissected, triturated and plated. The cultures were loaded with 100 nM ^{3}H glutamate, washed extensively and depolarized with a high K^+ solution. The cultures were electrophysiologically healthy and cells survived the experimental manipulations. Glutamate release was minimal from E13 to E17, then increased rapidly to a peak level ~two-fold greater than baseline at E19.5, and finally fell to minimal levels by E22.5. The effect of 5HT on glutamate release was tested by exposing cultures to 100 nM 5HT immediately before treatment with the high K^+ solution. Under these conditions, 5HT did not influence depolarization induced glutamate release. This work demonstrates that depolarization induced glutamate release is present prenatally and can be studied in a culture system of living, embryonic, cortical cells. Serotonin modulation of glutamate release was not detected.

489.12

THE EFFECT OF TRYPTOPHAN HYDROXYLASE INHIBITION ON SUBSTANCE P mRNA DEVELOPMENT IN THE MEDULLARY RAPHE NUCLEI. P.D. Walker, L. Ni*, T.L. Green*, S. Schotland*, R.P. Hart, and G.M. Jonakait. Dept. of Biol. Sci., Rutgers University, Newark, NJ 07102.

Substance P is co-localized with serotonin (5-HT) in almost all of the neurons developing in medullary raphe nuclei, B1 and B2 (Ni and Jonakait, Soc. Neurosci. Abst., 1987). Factors regulating co-localized neurotransmitter molecules during development are ill-defined. We sought to determine possible changes in SP mRNA levels following inhibition of tryptophan hydroxylase (TPH), the rate-limiting enzyme in 5-HT biosynthesis.

Pregnant rats received Alzet minipump implants containing *p*-chlorophenylalanine (*p*CPA) on day 8 of gestation (E8). Each pump contained an amount of *p*CPA sufficient to deliver 100 mg/kg/day for 14 days. In addition, a 300 mg/kg s.c. injection was given at the time of implantation. Control animals received sham surgery with no pump implantation. As measured by radioenzymatic assay, TPH activity in dorsal raphe nuclei from *p*CPA-treated litters was inhibited by 60-70% from E14 to post-natal day 3 (PND3) returning to control levels at PND 8. Total RNA isolated from medullary raphe nuclei of control and *p*CPA-treated litters was subjected to Northern blot analysis using a radioactive RNA probe for rat preprotachykinin (subclone from pGem 2-31-1, kindly provided by J. Krause, Washington University, St. Louis, MO) In contrast to TPH activity, SP mRNA levels were down to 7% of control at E14 and steadily increased to equal control levels from E19 to birth. At PND1, SP mRNA levels were increased four-fold and then fell to control levels by PND8.

These results suggest that inhibition of 5-HT biosynthesis has regulatory consequences for co-localized peptide neurotransmitters. (Supported by NS 23687. GMJ and RPH are Johnson & Johnson Discovery Research Fellows.)

CATECHOLAMINES VI

490.1

DOPAMINE (DA) SYSTEM RESPONSES IN THE AMYGDALA (AM) AND CAUDATE-PUTAMEN (CP) TO ACUTE VS. REPEATED REMOXIPRIDE TREATMENT IN THE RAT. E.C. Essig* and I.C. Kilpatrick* (SPON: P. Dean). Dept. of Pharmacology, Medical School, University Walk, Bristol BS8 1TD, England.

Current data suggest that an action of neuroleptic drugs on DA systems in non-CP loci may confer their anti-psychotic activity. Here, we report the influence of an atypical neuroleptic, remoxipride (RMX) on DA utilisation in AM and CP after both acute and chronic treatments.

RMX (2.1 mg/kg i.p.) or its vehicle was given to male Wistar rats (n=10-12 per group) as either single (acute) or repeated injections once daily for 10 days. Food and water were freely available. Two hours after the last dose, AM and CP were assayed for DA and its two major metabolites, DOPAC and HVA by an HPLC-EC method.

Acutely, RMX evoked large elevations in DA utilisation in both AM (DOPAC +59%, $p<0.001$; HVA +163%, $p<0.01$) and CP (DOPAC +232%, $p<0.01$; HVA +236%, $p<0.01$). After 10 days RMX treatment, these changes were less pronounced in CP (DOPAC +43%, $p<0.001$; HVA +75%, $p<0.01$). In the AM, the relative increase in HVA concentration was almost halved (+71%, $p<0.05$). A small elevation in DOPAC remained (+14%) but this was no longer significant.

These data suggest that the profound neurochemical 'tolerance' of DA systems seen in CP to repeated dosing with RMX can also be seen to a lesser degree in AM. In view of the report that meso-amygdaloid DA neurones do not appear to possess autoreceptors that regulate DA synthesis (Kilts et al., J. Neurosci. 7, 3961-3975), the present data may reflect an RMX-specific action.

We thank Astra Alab (Sweden) for supplies of RMX.

490.2

EFFECTS OF RISPERIDONE (RIS), A NEW NEUROLEPTIC, ON NIGRAL-STRIATAL DOPAMINERGIC (DA) NEURONS. P.S. Blum and C.B. Davis*. Department of Biological Research, Janssen, Research Foundation, Spring House, PA 19477.

RIS was compared to haloperidol (HAL) on DA neurons using two methods. A) apomorphine (APO)-induced inhibition of DOPA synthesis, and B) activity of DA neurons in the substantia nigra (SN). To measure DOPA synthesis, rats were treated with gamma-butyrolactone to block activity of SN neurons, and NSD-1015 to block DOPA decarboxylase. After treatment, striatal DOPA levels were 22.4+0.9 pmol/gm, about 10 times the levels in untreated animals (2.8+0.2 pmol/gm). DOPA levels were reduced 51% by APO pretreatment (1 mg/kg i.p.). Both RIS (ED_{50}=0.32 mg/kg i.v.) and HAL (ED_{50}=0.06 mg/kg i.v.) blocked APO-induced inhibition of DOPA synthesis. These data suggest that RIS, like HAL, is a presynaptic D-2 antagonist at the terminals of DA neurons. In separate experiments, recordings were made from DA neurons in the SN of chloral hydrate anesthetized rats. APO inhibited activity in these neurons (ED_{50}= 12+2 µg/kg i.v.), and HAL blocked this inhibition with a threshold dose of 20 µg/kg i.v. RIS, however, did not consistently block APO-induced inhibition of neural activity. Rather, RIS either had no effect (to 80 µg/kg i.v.) or produced an abrupt cessation of neural activity (between 80 and 320 µg/kg i.v.). Unlike HAL, and unlike the effect of RIS at the nerve terminal, RIS had no detectable effect at D-2 receptors in the SN.

490.3

EFFECTS OF CHRONIC HALOPERIDOL ADMINISTRATION ON POPULATIONS OF DOPAMINE NEURONS INNERVATING THE AMYGDALOID NUCLEAR COMPLEX AND BED NUCLEUS OF THE STRIA TERMINALIS (BNST). T. Ely*, C. Anderson*, C. Kilts*. (SPON:M. McMilian). Duke Univ. Med. Center, Durham, NC 27710.

The present study mapped the biochemically estimated response to chronic haloperidol administration of populations of dopamine (DA) neurons innervating discrete projection fields within the limbic system. Animals sacrificed 24 hr following the last of 28 daily haloperidol (HAL) treatments (0.3mg/kg, i.p.) exhibited a significant decrease in the HVA and DOPAC concentration of selective brain nuclei (e.g. olfactory tubercle, cingulate cortex). Acute HAL administration significantly increased the HVA and DOPAC concentration of all brain nuclei examined, particularly the ventral and dorsal subdivisions of the BNST. Upon accounting for the residual effect of chronic HAL administration on DA metabolism, a significant attenuation of the biochemically estimated response to HAL following chronic HAL administration was noted in only a minority of brain nuclei. DA neurons projecting to the central and lateral amygdaloid nuclei exhibited the greatest degree of tolerance development while the response to HAL of neurons projecting to the intercalated cell groups and cortical amygdaloid nucleus was not influenced by chronic drug administration. These data further reinforce the pharmacological heterogeneity of mesoamygdaloid DA neurons and suggest their inequivalent role in the mechanism of antipsychotic drug action. (NIMH MH 39967).

490.4

HALOPERIDOL FORMS MOLECULAR COMPLEXES WITH DOPAMINE IN VIVO. T. A. Patterson* and J. O. Schenk. Dept. of Chemistry and Programs of Biochemistry and Pharmacology/Toxicology, Washington State University, Pullman, WA 99164-4630.

A previous report from this laboratory suggested that the dopamine (DA) antagonist haloperidol (HAL) forms molecular complexes with DA in vitro (see Neurosci Abst. 13: 134.8, 1987). Herein we report that in vivo voltammetric studies suggest that these complexes also form in vivo.

Experiments were conducted using 300 um diameter carbon paste sensing electrodes in order to obtain optimal voltammetric peak shapes. An injection syringe was placed ca. 1 mm from the sensing electrode and the electrode assembly placed in the striatum of a chloral hydrate anaesthetized rat. Analysis was made by linear potential sweep voltammograms run every 10 minutes from 0.00V to 0.50V, scan rate = 10 mV/sec. A baseline oxidation potential was established for DA by direct injection of dopamine, 6.65 pmole of HAL was injected and the oxidation peak was again monitored every 10 minutes for at least 70 minutes. The injection of HAL results in a shift of the DA oxidation potential to higher values, indicating that a complexation reaction has occurred. This shift also occurs following a systemic injection of HAL (2 mg/kg). Supported by: MH42759 and the State of WA.

490.5

DIFFERENTIAL REGULATION OF MESOTELENCEPHALIC DOPAMINE NEURONS BY THE SEROTONERGIC AGONIST 8-HYDROXY-DPAT. A. M. Rasmusson, R. H. Roth, and A. Y. Deutch. Depts. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

The selective stress-evoked activation of the dopamine (DA) neurons innervating the prefrontal cortex (PFC) does not appear attributable to a lack of impulse-modulating autoreceptors on these neurons: other DA neurons which also lack these autoreceptors are not activated by mild footshock stress. These data suggest that extrinsic controls over DA neurons may be critical to the regulation of central DA systems. The serotonin innervation of the midbrain DA neurons may be such a control system. We have examined the effects of the serotonin agonist 8-hydroxy-DPAT, which at low doses is an autoreceptor agonist, on both basal activity and stress-evoked activation of the mesotelencephalic DA systems. DA turnover, as reflected by DOPAC/DA, was significantly increased in the PFC by 225 ug (but not 75 ug) 8-OH-DPAT; DA turnover was not altered in the nuc. accumbens or striatum. The stress-induced activation of mesoprefrontal DA neurons was essentially unaltered by 8-OH-DPAT. However, the high dose of 8-OH-DPAT resulted in a stress-induced increase in NAS DA turnover. These data suggest that 5-HT mechanisms may differentially regulate the mesotelencephalic DA subsystems.

490.6

DOPAMINE METABOLISM IS SELECTIVELY ALTERED IN PREGNANCY. R.E.A. Craig* and C.E. Greenwood. Dept. Nutr. Sci., Fac. Med., Univ. of Toronto, Toronto, Ontario, Canada. M5S 1A8.

The relationship between acute administration of precursor amino acids (AA) and monoamine (MA) metabolism has been well elucidated in adult rats. However, the effect of chronic AA administration on NT metabolism in altered physiological states is less well understood. Therefore, to examine the effect of AA supplementation on MA metabolism during pregnancy, 20% casein diets, enriched with 5% L-phenylalanine (PHE) or equimolar tyrosine (TYR), were fed to pregnant (PREG) rats from G12-G20 and to nonpregnant (NPREG) rats for 9 days. Of the MAs, only dopamine (DA) metabolism was altered during pregnancy. Despite similar brain TYR levels, steady-state levels of DOPAC, the major metabolite of DA, were reduced during pregnancy in striatum (1608±75 vs 1118±43 ng/g; X±SEM for NPREG and PREG rats) and remaining hemispheres (110±7 vs 85±4 ng/g). Hypothalamic DA was lowered (401±40 vs 277±16 ng/g) in PREG rats; DOPAC remained unchanged. Similar effects of pregnancy were seen when DA and metabolites were measured following haloperidol injection (2 mg/kg, i.p.) and when DA synthesis was estimated using NSD 1015 (100 mg/kg, i.p.). Feeding precursor AA elevated DA in PREG rats to NPREG levels. No effect of pregnancy on steady-state levels of either norepinephrine or serotonin and its metabolite 5HIAA was observed. The results indicate that DA metabolism is decreased in pregnancy, and that this effect can be alleviated by dietary AA supplementation. (NSERC).

490.7

REGIONAL AND SIDE DEPENDENT EFFECTS ON DOPAMINE UTILIZATION INDUCED BY DIFFERENT DURATIONS OF RESTRAINT STRESS. J.N. Carlson, S.D. Glick and J.L. Baird*. Department of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208.

We have studied the effects of restraint (immobilization) stress on dopamine (DA) utilization in projection areas of the mesocortical (prefrontal cortex - PFC), mesolimbic (nucleus accumben -NAS), and nigrostriatal (striatum - STR) systems. Female Long-Evans rats were restrained in Plexiglas cylindrical tubes for 15 minutes, 30 minutes or 60 minutes, and then immediately decapitated. Bilateral samples of the above brain regions were removed and assayed for DA, DOPAC and HVA content by HPLC with electrochemical detection. At 15 minutes, DA utilization, as indicated by HVA/DA and DOPAC/DA was elevated in the left PFC. At 30 minutes these measures were elevated on both sides of the PFC with the left side being greater; at 60 minutes, these measures declined bilaterally, although the left side was still higher than the right. The NAS was bilaterally elevated at 30 minutes and at 60 minutes. There was little effect of any duration of restraint in the striata. The data suggest that, as stress is prolonged, different DA systems, as well as different sides of the PFC, are sequentially activated. (Supported by NIDA grant DA03817 to S.D.G.).

490.8

EFFECTS OF DOPAMINE DEPLETION OF THE PREFRONTAL CORTEX ON STRESS-INDUCED CHANGES IN MESOLIMBIC AND STRIATAL DOPAMINE FUNCTION. W. A. Clark, R. H. Roth, and A. Y. Deutch. Depts. of Pharmacology and Psychiatry, Yale Univ. School of Medicine, New Haven, CT 06510.

Mild footshock stress and conditioned fear selectively increase dopamine (DA) metabolism in the prefrontal cortex (PFC); more severe stress recruits other DA neurons, such as those projecting to the nucleus accumbens (NAS). It is unclear whether DA release in the PFC is a permissive event (initiator) allowing other catecholamine neurons to respond, or whether PFC DA release is part of a coping response. Previous data indicating that DA autoreceptor-selective doses of apomorphine are anxiolytic favor an initiator role for PFC DA release. We have therefore examined the effects of stress on DA systems in the rat NAS and striatum (CP) after 6-hydroxydopamine lesions of the PFC. A stress-evoked increase in the DA metabolite DOPAC was observed in the NAS, but not CP. Lesions of the DA innervation of the PFC blocked the stress-induced increase in DA metabolism in the NAS, but did not significantly alter DOPAC levels in the CP. These data suggest that the preferential activation of mesoprefrontal DA neurons evoked by stress may be necessary for the subsequent involvement of other catecholamine neurons which contribute to means of coping with stress.

490.9

TURNOVER OF CENTRAL BIOGENIC AMINES IN THE RAT DURING ACUTE AND CHRONIC COLD EXPOSURE. A.Y-C. Shum*, C-F. Chen* F-Y. Liao* and J-Y. Wang (SPON: H-S. Yin). Dept. of Pharmacology, National Yang-Ming Medical College, and Dept. of Physiology, National Defense Medical Center, Taipei, Taiwan, R.O.C.

Turnover rates provide valid indices of neuronal activities. A non-isotopic, non-steady state method for estimating amines turnover was applied to the investigation of the possible involvement of monoaminergic neurons during cold exposure and adaptation in the rat, a homeotherm. Male Sprague-Dawley rats were cold exposed (4°C) for 30 mins, 24 hrs, 1 and 6 weeks respectively and the turnover of catecholamines and serotonin in different brain regions estimated from the acumulation of the intermediates dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5HTP) after inhibition of the common synthesizing enzyme l-aromatic acid decarboxylase. Increased metabolism, as reflected by increase in oxygen consumption, was observed after 1 and 6 weeks of exposure. Proliferation of mitochondria in the interscapular fad pad, thereby turning it from white to brown, was also observed. These observations indicated the shifting of mode of thermogenesis from shivering to nonshivering. Coinciding with these changes, increased turnovers of serotonin and catecholamines were also observed in the anterior hypothalamus, midbrain-pons, and medulla oblongata. These observations suugest strongly of physiological involvement of central monoaminergic neurons in cold acclimation.

490.10

EFFECTS OF MAO-B INHIBITOR ON DOPAMINE SYSTEMS IN MPTP TREATED MICE. B.K. Gupta and M. Gupta, Department of Anatomical Sciences and Neurobiology, School of Medicine, Univ. of Louisville, Louisville, KY 40292

Our previous studies have shown that treatment of mice with MPTP decreases dopamine levels in the striatum as well the number of fluorescent cell bodies in the substantia nigra pars compacta (SN). Furthermore MPTP neurotoxicity can be prevented by pre-treatment with MAO-B inhibitor. MAO-B activity in the brain increases with age which leads to decreased catecholamines. The present studies were undertaken to investigate if treatment of MPTP lesioned mice with MAO-B inhibitor, deprenyl, prevents further deterioration of the nigrostriatal dopamine system and other systems in the brain. Male C57BL/6 mice at 18 months of age were divided into four groups: (a) control, (b) MPTP treated at 60 mg/kg given in multiple injections over two days, (c) MPTP followed by deprenyl three days later, (d) deprenyl alone (0.035 mg/5ml in drinking water for upto 10 days. All the four groups of animals were sacrificed by decapitation, brains were quickly dissected out and frozen on dry ice. 16 um thick frozen sections were cut using a cryostat and processed for fluorescence histochemistry using the SPG method. Our results show a decreased number of fluorescent neurons in the SN with MPTP treatment as compared to the controls and were quantitated in the four groups. Results of these studies will be presented in details. Supported by USPHS grant R29 NS 24291 to MG.

490.11

DOPAMINE AND TYROSINE HYDROXYLASE LEVELS IN THE CORTICAL AND SUBCORTICAL AREAS IN THE MPTP-PARKINSONIAN MONKEYS. I.J.Kopin, A.Zuddas, V.Weise*,R.J.Plunkett*, E.H.Oldfield* and K.S.Bankiewicz,(SPON:R. Fox) NIH, NINCDS, Bethesda, MD, 20892

Dopamine (DA) and tyrosine hydroxylase (TH) were measured in brain areas of one full parkinsonian, three hemiparkinsonian (HPD) and two normal monkeys. Cortical areas of interest were: prefrontal cortex, medial frontal gyrus, frontal eye field, motor cortex, superior temporal sulcus, parietal sulcus, parietal gyrus, occipital gyrus, cingulate gyrus. Subcortical areas were: dorsal and ventral caudate nucleus, dorsal and ventral putamen, head of caudate nucleus, nucleus accumbens, superior colliculus and inferior olive. DA and TH in cortex of hemiparkinsonian monkeys were similar on both the MPTP infused and contralateral sides and not significantly different from controls. However, the full parkinsonian animal showed elevated cortical levels of dopamine. DA and TH in the caudate and putamen in HPD on the MPTP treated side and in the full parkinsonian monkey were decreased >95% when compared to non-treated side in HPD or control monkeys. Caudate and putamen in the non-MPTP treated side in HPD did not differ from controls. Other subcortical areas showed some decrease in DA and TH levels in the full parkinsonian animal and on the MPTP treated side in HPD monkeys. These results further support the specificity of the effects of MPTP on dopaminergic neurons in the nigrostriatal pathway and indicate that the delays in initiation of motion are not due to deficit in cortical dopamine.

490.12

THE ROLE OF THE DOPAMINE TRANSPORT SYSTEM IN MPTP-INDUCED MITOCHONDRIAL DAMAGE. S. Ofori*, R.E. Heikkila and W.J. Nicklas. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The administration of MPTP or MPTP analogs, such as 2'CH_3-MPTP, to experimental animals leads to the destruction of dopaminergic neurons of the nigrostriatal pathway. Neurotoxicity is dependent upon the monoamine oxidase catalyzed formation of the pyridinium species (MPP^+ or 2'CH_3-MPP^+) from the tetrahydropyridine, and selective uptake of the pyridinium species into dopamine (DA) neurons by the DA transport system. Pretreatment with inhibitors of DA uptake protect against neurotoxicity. In experiments with neostriatal slices, the pyridinium ions formed from MPTP and 2'CH_3-MPTP inhibited complex 1 of mitochondrial electron transport, and thereby increased lactate (LAC) production. DA uptake inhibitors (i.e. Win 35,428, Mazindol and McN 5908) reduced 10μM 2'CH_3-MPTP-induced LAC formation by 57%, 45% and 34% respectively. Win 35,428 lowered 5μM-induced LAC accumulation by 82%, and that due to 50μM MPTP by 45%. Correlations will be made between the capacity of uptake inhibitors to decrease the tissue content of the pyridinium species, and LAC formation. These results are consistent with the concept that toxic effects of MPTP and 2'CH_3-MPTP are due to uptake of the pyridinium species into the DA neuron, and subsequent mitochondrial damage.

490.13

DEVELOPMENT OF THE DOPAMINERGIC NIGROSTRIATAL PATHWAY IN MALE SWISS-WEBSTER MICE: RELATIONSHIP TO MPTP-INDUCED NEUROTOXICITY. L. Manzino, D. Tonzola*, P.K. Sonsalla, B.A. Sieber*, A. Giovanni, and R.E. Heikkila. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

MPTP-induced dopaminergic neurotoxicity depends on 1) the monoamine oxidase (MAO) B dependent formation of the pyridinium species MPP^+ and 2) the uptake of MPP+ by the dopamine (DA) transport system. Aged animals are known to be more sensitive to MPTP than young adults whereas immature animals are less sensitive. One possible explanation for these sensitivity differences is that brain MAO-B activity increases during aging. In developing mice, we have measured neostriatal levels of MAO-B and MAO-A as well as biochemical markers of dopaminergic activity (tyrosine hydroxylase activity, ^{3}H-DA uptake, DA content). The development of MAO-A activity was considerably more rapid than that of MAO-B activity. The development of neostriatal dopaminergic markers paralleled that of MAO-B. Since MPTP-induced toxicity is dependent on MAO-B and the DA transport system, any differential effects of MPTP during development might be due to either parameter. We will discuss relationships between: 1) MPTP-induced toxicity, 2) toxicity produced by 2'ethyl-MPTP (which is bioactivated by MAO-A and 3) the development of MAO activity and the nigrostriatal dopaminergic pathway.

490.14

EFFECTS OF MPTP, 2'ETHYL-MPTP AND THEIR PYRIDINIUM SPECIES ON CULTURED PC-12 CELLS. A. Basma*, H. Geller and R.E. Heikkila. Depts. of Neurology and Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The dopaminergic neurotoxicity of MPTP is dependent upon the monoamine oxidase-B (MAO-B) catalyzed oxidation of MPTP to a dihydropyridinium intermediate which in turn is oxidized to the pyridinium species MPP^+. In the present study PC-12 cells ($50x10^3$) were exposed to varying concentrations of MPTP, 2'ethyl-MPTP or their pyridinium species for periods of 1-14 days. Toxicity was assessed by resuspending live cells and counting aliquots. The PC-12 cell line contains high levels of dopamine and MAO-A, but relatively little MAO-B. The MPTP analog, 2'ethyl-MPTP, which is considerably better than MPTP as a substrate for MAO-A, was much more toxic than MPTP in PC-12 cells. Both the toxicity of MPTP and 2'ethyl-MPTP and the formation of their corresponding pyridinium species were largely attenuated by the MAO inhibitor pargyline. The pyridinium species themselves, MPP^+ and 2'ethyl-MPP^+, were relatively equipotent and quite toxic to PC-12 cells. These data suggest that the MAO-A catalyzed formation of pyridinium species from MPTP and 2'ethyl-MPTP plays an important role in the toxicity to PC-12 cells. This cell line represents an excellent model system with which to study the mechanism of action of MPTP.

490.15

DIFFERENTIAL EFFECTS OF MPTP IN THE RAT AND MOUSE. A. Giovanni, P.K. Sonsalla and R.E. Heikkila. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

MPTP administration causes a degeneration of the nigrostriatal dopaminergic pathway in mice. In contrast, rats are considerably less sensitive. In the present study, we administered MPTP to male Sprague-Dawley rats (Charles River) and male Swiss-Webster mice (Taconic Farms) and measured brain levels of MPTP and its major metabolite 1-methyl-4-phenylpyridinium (MPP^+). In contrast to what is generally accepted, we found similar levels of MPP^+ in the brains of rats and mice over a 2 hour time period after a single s.c. injection of 40 mg/kg of MPTP. In parallel experiments, MPTP administration caused a dopamine depletion of approximately 70% in the mouse neostriatum but had no significant effect in the rat neostriatum. These data suggest that the differential sensitivity of rats and mice to MPTP is due to more than simply the brain level of MPP^+. We have devised different dosing regimens in an attempt to maintain high brain levels of MPTP and MPP^+ for extended time periods. Experimental variables included: the dose of MPTP, the number of injections, and the interval between injections. By altering the above variables we have been able to obtain a substantial depletion of neostriatal DA in the rat. Reasons for the differential effects of MPTP in the rat and the mouse will be discussed.

490.16

(+)MK-801 PROTECTS AGAINST METHAMPHETAMINE (METH)-INDUCED, BUT NOT MPTP-INDUCED, NEUROTOXICITY IN MICE. P.K. Sonsalla, W.J. Nicklas and R.E. Heikkila. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

METH or MPTP administration to mice damages nigrostriatal dopaminergic neurons. Toxicity induced by METH appears to depend on DA, whereas that induced by MPTP depends on formation of its metabolite, MPP^+. Although the exact cellular mechanisms responsible for cell death by METH or MPTP are unknown, oxidative stress has been implicated for both. It has been suggested that oxidative stress associated with massive release of excitatory amino acids (EAAs), i.e. glutamate, might underlie reperfusion or postischemic neurodegeneration. Reperfusion damage can be prevented by (+)MK-801, a non-competitive antagonist of the N-methyl-D-aspartate receptor. In the present studies, we found that (+)MK-801 prevented METH-induced, but not MPTP-induced, dopaminergic neurotoxicity. In mice treated with METH, neostriatal DA content and tyrosine hydroxylase activity were approximately 50% of control values; administration of (+)MK-801 prior to and after METH prevented these decrements. These results suggest that oxidative stress mediated by EAAs may play a role in the neurotoxic actions of METH, but not those of MPTP. These observations may have implications for several neurodegenerative disorders where oxidative stress might be involved in pathogenesis of the disease.

NEUROTOXICITY IV

491.1

MPP^+ IS NEUROTOXIC TO CEREBELLAR GRANULE CELLS. A. Marini*, T. S. Nowak, Jr. and I. Kopin (SPON: K. M. Newrock) CNB and LNNS, NINCDS, NIH, Bethesda, MD 20892

We have developed a neuronal culture system to study the neurotoxicity of MPP^+. Cerebellar granule cells are susceptible to the neurotoxic effects of MPP^+ at 50-100uM, evident as a loss of neuronal processes and disintegration within several days. In contrast, MPTP is not toxic. In cocultures of cerebellar astrocytes and granule cells MPTP is selectively neurotoxic to the granule cells, and this toxicity is prevented by the addition of the MAO inhibitor pargyline. Preliminary results in pure granule cell cultures indicate that MPP^+ at 30 uM or more results in a striking reduction of phosphocreatine (PCr) in the absence of significant ATP loss. The decrease in PCr was evident within 3 h after addition of MPP^+, and preceded any morphological evidence of neurotoxicity. Our results suggest that these culture systems are suitable for studying several aspects of the neurotoxic effects of MPTP and MPP^+, including components of uptake and metabolic conversion. The biochemical evidence that PCr levels fall prior to any effect on ATP levels or overt cell death is perhaps consistent with reports of mitochondrial dysfunction following exposure to MPP^+ in vitro. Future studies may be expected to further define mechanisms by which MPP^+ affects neuronal energy metabolism and to determine the relevance of such effects to its neurotoxicity.

491.2

ASTROCYTES INVOLVEMENT IN N-METHYL-4-PHENYL-1,2,3,6 TETRAHYDROPYRIDINE NEUROTOXICITY. G. Cappelletti*, G. Malanca*, A. Vescovi* and E.A.Parati.Inst. Neurol. "C.Besta" Milan, Italy, 20133.

Neuroglial cells seem to be involved in toxic mechanism of the potent drug N-Methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP). MPTP toxicity is probably mediated by its toxic metabolite MPP+, through the action of monoamine oxidase B (MAO-B). In the brain, MAO-B is contained predominantly in glial cells. Moreover the "in vitro" study directly showed that pure cultures of mammalian astrocytes are capable of converting MPTP to MPP+. In order to better understand the glial cells role in promoting neuronal degeneration we studied the correlation between the metabolic properties of cultured rat cerebellum astrocytes and the survival of cultured PC12 cells,considered as a model of dopaminergic neurons. We exposed astrocytes to different MPTP concentrations (0.1-1 μM), evaluated cell viability and collected the conditioned media at several incubation time. Preliminary data showed the conditioned medium of MPTP-treated astrocytes seems to be more toxic on PC12 cells than non-conditioned medium containing similar MPTP and MPP+ concentrations. We discuss these data in terms of astrocytes involvement in MPTP neurotoxicity.

491.3

DIETHYLDITHIOCARBAMIC ACID ENHANCES THE NEUROTOXICITY OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE IN THE CD-1 MOUSE. D.B. Miller, J.P. O'Callaghan and J.F. Reinhard, Jr.[1] Health Effects Rsch. Lab., U.S. EPA and [1]Wellcome Rsch. Labs., Burroughs Wellcome Co., RTP, NC 27711.

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a catecholaminergic (CA) neurotoxicant in man, nonhuman primates and certain mouse strains. Diethyldithiocarbamic acid (DDC) increases CA depletion and the glial response in C57Bl/6 mice. Here we report on DDC (400 mg/kg i. p.) and MPTP (5, 10 or 20 mg/kg i. p. X 4 every 2 hrs) in female CD-1 mice, a nonsensitive strain. To be neurotoxic MPTP must be metabolized to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium (MPP+). To partially eliminate kinetic and metabolic factors the effect of DDC after ICV MPP+ (10 or 20 ug) and on bovine adrenal medullary (BAM) cells in culture (0.3 mM MPP+ and 0.3 - 3.0 mM DDC) was also examined. DDC increased the general toxicity of MPTP and increased CA depletions at both 5 and 10 mg/kg MPTP. DDC also increased the toxicity of MPP+ in BAM cells as evidenced by decreased CA levels and increased total protein. ICV MPP+ toxicity was also increased. It appears DDC can enhance the neurotoxicity of MPTP and MPP+ in nonsensitive mouse strains.

491.4

CHARACTERIZATION OF MPP+ TOXICITY FOR RAT DOPAMINERGIC NEURONS IN CULTURE. P.P. Michel*, H. Zawadcka*, M. Goldstein, J. Sanchez-Ramos, W.L. Strauss and F. Hefti. Depts. Neurology and Pharmacology, Univ. of Miami, Miami, FL 33101, and Neurochemistry Research Unit, NYU Medical Center, New York, NY 10016.

In cultures of dissociated fetal rat mesencephalic neurons, MPP+ and its analog 2'-methyl-MPP+ selectively affect dopaminergic neurons. 10μM MPP+ during 48 hours reduced the number of tyrosine hydroxylase (TH)-positive neurons by 90% but failed to reduce the number of TH-negative cells. The number of TH-positive neurons declined further, when sister cultures were kept after treatment for up to 6 days in MPP+-free medium. 10μM MPP+ for 48 hours reduced the uptake of 3H-dopamine by 95%. There was no recovery when cultures were kept in MPP+ free medium for another 6 days after the treatment. These findings, together with earlier morphological observations, strongly suggest that MPP+ results in morphological destruction of the cultured rat dopaminergic neurons. Using Northern blotting and in situ hybridization techniques, we presently are investigating the effects of MPP+ treatment on levels of mRNA[TH].

491.5

MPP+ RESISTANT PC12 MUTANTS CREATED BY RETROVIRAL INSERTION. M.J. Kadan and M.M.S. Lo. NIDA,ARC, Baltimore, MD 21224.

The catecholaminergic cell line, PC12, provides a useful model system in which to study the mechanism of 1-methyl-4-phenylpyridinium (MPP^+) neurotoxicity. Infection of PC12 cells by retroviruses creates random mutations throughout the genome. MPP^+ resistant mutants resulting from retroviral insertion can be used to identify the genes involved in MPP^+ neurotoxicity.

We have infected PC12 cells (10^8) with a recombinant retrovirus pZIPNEOSV(X). These infected cells were selected for expression of virus and resistance to 500μM MPP^+. We have determined independently 1) frequency of infection ($1x10^{-4}$), and 2) frequency of spontaneous mutations resulting in MPP^+ resistance ($1.4x10^{-4}$). The number of virally infected MPP^+ resistant colonies we obtained ($1x10^{-6}$) is approximately 100 times greater than the number predicted if these events (viral infection and MPP^+ resistance) are unrelated. This shows a significant correlation between viral intergration within the selected mutants and induction of a mutation resulting in resistance to MPP^+. We have obtained a large library of MPP^+ resistant clones. Analysis of the proviral positions within these mutants will allow us to identify and characterize the gene targets involved in MPP^+ neurotoxicity.

Supported in part by a grant from Johns Hopkins C.A.A.T.

491.6

CHRONIC TWO-FOLD VITAMIN E (VE) SUPPLEMENTATION ANTAGONIZES MPTP-INDUCED NEUROLOGICAL AND NEUROCHEMICAL DEFICITS IN CATS. J.S.Althaus*, M.A.Travis, E.D.Hall and P.F.VonVoigtlander. CNS Diseases Research Unit, The Upjohn Company, Kalamazoo, MI 49001

The effects of a two-fold increase in dietary VE (from 35 to 70 I.U./lb of dry food) for 12 weeks were examined on the dopaminergic neurotoxicity of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in cats. Neurological dysfunction was quantified by blinded evaluators using a 6-parameter scale and dopamine (DA) and homovanillic acid (HVA) were measured in the ventral limbic, tegmental and caudate regions via HPLC with electrochemical detection. Cats were treated with 0.9% saline or MPTP (5 mg/kg) i.p. once daily for 3 days and neurological and neurochemical evaluation carried out on the first post-treatment day. Control saline-treated, normal VE cats (n=5) had a mean neurological assessment score (maximum = 14) of 12.1 ± 0.5 (x ± S.E.M.) while normal VE cats treated with MPTP (n=4) had a score of only 6.5 ± 0.7. In contrast, MPTP treated, VE supplemented animals (n=13) had a mean score that was significantly improved to 9.9 ± 0.4. Correlated with the neurological dysfunction, the normal VE, MPTP-treated cats showed significant depletions of DA in all three brain regions examined, whereas in the VE supplemented, MPTP-treated cats, significantly higher levels were measured in the caudate and ventral limbic areas. These results show a clear protective effect of the anti-oxidant VE against MPTP neurotoxicity.

491.7

1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) SELECTIVELY DEPRESSES BRAIN BUFARALOL HYDROXYLASE ACTIVITY IN C57 BL/6J MICE. G.S. Shahi*, N.P. Das+*, E.J.D. Lee°* and S.M. Moochhala°†SPON: P.T-H. Wong) Depts of Physiology, +Biochemistry and °Pharmacology, Faculty of Med., National Univ. of S'pore, Singapore 0511.

MPTP is a neurotoxin which causes an irreversible parkinsonian syndrome clinically indistinguishable from idiopathic Parkinson's disease. It can be detoxified by metabolism via the cytochrome P-450 enzyme system (Weissman et al, J.Med.Chem., 28:997-1001, 1985). Bufaralol hydroxylase (buf) is thought to be the cytochrome P-450 enzyme activity capable of detoxifying MPTP in the brain (Fonne-Pfisher et al, Biochem. Biophys. Res. Commun, 148: 1144-1150, 1987).

We have studied the in vivo effects of MPTP on buf and aryl hydrocarbon hydroxylase (AHH) activities in mouse brain. We found that MPTP (given i.p.) inhibited buf and AHH activities in a dose-dependent manner. MPTP appears to selectively depress buf activity and is about 2300 times more potent in depressing buf activity compared to AHH activity (ID_{50} for buf $1x10^{-4}$mg/kg and ID_{50} for AHH $2.3x10^{-1}$ mg/kg). In vitro studies were also carried out.

The possible clinical implications of our findings with respect to Parkinson's disease will be discussed.

491.8

RETINAL DOPAMINE SENSITIVITY TO MPP^+ TOXICITY. C Harnois, T Di Paolo, G Marcotte* & M Daigle*. Centre Hospitalier de l'Université Laval, Département d'ophtalmologie et École de pharmacie, Université Laval, Québec, PQ, Canada.

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is not metabolized into active 1-methyl-4-phenylpyridinium (MPP^+) in the eye[1], and does not decrease retinal dopamine content in monkeys[2]. MPP^+ was injected into the vitreous of rabbit eyes; one eye received doses of either 7, 70 or 700 μg, the other being used as control. Electroretinograms (ERG) and oscillatory potentials (POS) were recorded. DA and its metabolites were analyzed by HPLC-EC.

Two eyes treated with 700 μg presented extinguished ERG and POS from day 1 to day 44 and a decreased retinal DA concentration (15% of control on day 1 and 40% on day 31). Injection of 7 μg of MPP^+ had no effect on the ERG, the POS or the retinal DA concentration. The eyes treated with 70 μg showed different results. On day 1, the b-wave of the ERG was selectively decreased (15% of the control) and the a-wave was not affected, suggesting retinal drug toxicity. On day 21, the ERG and POS were about 40% of that of the control eye. On the other hand, DA concentration was 80% on day 1 and 114% on day 21. Fundus photographs and fluorescein angiographies revealed that eyes treated with either 700 or 70 μg of MPP^+ presented generalized retinal pigment epithelium modifications.

These data suggest that intravitreal injection of MPP^+ results in an unspecific retinal toxicity, and that it cannot be used to study the physiological role of retinal DA.

Supported by the Medical Research Council of Canada grants to CH and TDP.

[1] Langston et al, Neuroscience Letters 48: 87-92, 1984.
[2] Harnois et al, Invest Ophthalmol Vis Sci (suppl) 28:351, 1987.

491.9

MPTP DOSE-DEPENDENT LOSS OF MOUSE DOPAMINERGIC AMACRINES. W. Tatton, M. Kwan*, M. Verrier, N. Seniuk* and E. Theriault. Playfair Neuroscience Unit, The Toronto Hospital and Department of Physiology, University of Toronto, Toronto, Canada M5T 2S8.

MPTP produces dose dependent loss of neurons in substantia nigra compacta (SNC), locus coeruleus (LC), A13 hypothalamus and ventral tegmental area of 8-10 week old C57 black mice (Seniuk and Tatton, 1986). To investigate possible cell loss in retinae, C57 mice were given intraperitoneal MPTP according to two schedules: 30 mg/kg/day for 10 days (300 mg/kg total) or 30 mg/kg twice in one day (60 mg/kg total). Twenty days after the last dose the retinae were incubated with antiserum to tyrosine hydroxylase (TH) or choline acetyltransferase (CHAT) as whole mounts or serial sections. Normal retinae revealed TH and CHAT amacrines (AMs) in regular arrangements with respective mean densities of 43.8 ± 6.4 and 832 ± 20.3 cells/mm^2. The AM somas and terminal locations corresponded to those previously described for rabbit retinae (Famiglietti and Tumosa, 1986; Dowling and Ehinger, 1978). MPTP reduced TH AM densities to 7.4 ± 6.2 and 17.4 ± 8.1 for 300 and 60 mg/kg total doses. AMs in MPTP retinae did not show decreased TH staining intensities relative to normals and were randomly lost with no predilection for the central or peripheral retinae. TH AM loss was relatively greater for low MPTP doses than that for SNC and LC neurons. The alterations in visual contrast sensitivity (Bodis-Wollner et al., 1987) and PERGs (Nightengale et al., 1986) in Parkinsonian humans and the PERG abnormalities in MPTP-treated monkeys (Onofrj et al., 1986) may be at least partially explained by a reduction in dopaminergic amacrines' contributions to retinal processing (see Jensen and Daw, 1988). Supported by MRC grant MT5218.

491.10

NON-PARKINSONIAN TOXICITY OF 1-METHYL-4-PHENYL-1,2,5,6-TETRAHYDROPYRIDINE (MPTP): GASTRIC AND DUODENAL ULCERS. A. KESHAVARZIAN*, A. WIBOWO*, J.H. GORDON AND J.Z. FIELDS (SPON: I. Held). Dept. Med., Loyola Univ. Med. Sch., Maywood, IL 60153, and Med. & Res. Svces., Hines VA Hosp., Hines, IL 60141.

Although MPTP causes dopaminergic damage with subsequent development of parkinsonism, in rodents it also affects other neurotransmitter systems and produces other toxicity, namely, duodenal (DU) and gastric (GU) ulcers; the ulcerogenic mechanism is unknown. Since gender and age influence the metabolism of both neurotransmitters and toxins, we evaluated their influence on MPTP toxicity. MPTP-HCl [MPTP] sc, tid, was given to rats for 4 days and ulcers were checked on day 5. In 3 mo old female rats MPTP up to 15mg/kg/injection caused few ulcers [<30%]. Doses of 24 to 36 mg caused > 90% DU but > 50% mortality. MPTP at 20 mg/kg produced > 90% DU, 61% GU with < 20% mortality. Aging increased both mortality and the incidence of DU but not GU in female rats: [MPTP=15 mg, 3 mo=25% DU, 78% GU; 6 mo=33% DU, 100% GU; 12 mo=60% DU, 66% GU; 18 mo=66% DU, 66% GU]. Females had more DU (but not GU) than males. This difference disappeared after ovariectomy (OVX) [MPTP=20 mg, female=92% DU, 62% GU; male=42% DU, 58% GU; OVX=67% DU, 70% GU]. These observations suggest that both age and sex hormones are important in MPTP toxicity and may be due to their effects on MPTP metabolism to toxic metabolites (e.g. MPP+).

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491.11

Temporal Changes in Dopamine in Rats with MPTP induced Motor Deficits. M.E. Melnick and M.K. Shellenberger. Dept of Physical Therapy Education and Dept of Pharmacology. University of Kansas Medical Center, Kansas City, Ks 66103

We have previously reported changes in locomotor activity and gait patterns following treatment with MPTP in rats. The alterations in gait included a shortening of the stride, an increase in width and an increased tendency to walk with a flat-footed gait. The purpose of this project was to investigate the changes in dopamine (DA), norepinephrine (NE) and serotonin (5HT) concentrations in frontal cortex, striatum and substantia nigra 30, 60 and 90 days after injection of MPTP or solvent. Year-old rats were injected with MPTP or solvent (20 mg/kg,ip) for 7 days. Brains were removed 30, 60 or 90 days after the last MPTP injection and quickly frozen. Subsequently the brains were regionally dissected and prepared for chemical analysis using HPLC with electrochemical dissection. At 30 days post-injection DA levels in the striatum were 45% of control; at 60 days, 65% of control and at 90 days 72% of control. DA levels in the substanta nigra were not significantly reduced at any of the time periods investigated. These data indicate that there is some recovery of DA levels in the striatum with time following chronic administration of MPTP in the rat. Recovery of gait abnormalities lags behind the recovery of DA levels. We are presently evaluating the extent of gait abnormality with DA levels to determine the correlation between the behavior and neurochemical changes. (USPHS Grant NS 22124)

491.12

DOPAMINERGIC NEURONS IN THE SUBSTANTIA NIGRA IN MPTP LESIONED YOUNG AND AGED MICE. M. Gupta, Dept. of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, KY 40292.

Our previous studies have shown that young adult mice treated with MPTP demonstrate a reduced number of fluorescent neurons in the substantia nigra pars compacta (SN). Furthermore, aged mice treated with MPTP show marked physical symptoms of motor dysfunction followed by decreased number of fluorescent cell bodies in the SN. The present studies were undertaken to investigate if these severe physical symptoms seen in the MPTP treated aged mice are due to a more severe damage to the nigrostriatal system as compared to the young adult group. 3 and 21 months old male C57BL/6 mice were given multiple injections of MPTP (total dose 60-90mg/kg body weight) over a two day period. Three days after the last injection, control and treated animals were anesthetized with chloropent and perfused intracardially with saline followed by 4% paraformaldehyde. Brains were dissected out, frozen 40um thick serial sections were cut and alternate sections stained immunocytochemically for tyrosine hydroxylase (TH). Our results show a significant decrease in the number of TH-positive neurons in both the MPTP treated groups compared to their controls. Whether the TH-positive neurons are more significantly decreased in aged mice will be presented in details. Supported by R29 USPHS grant NS24291.

491.13

THE EFFECT OF MPTP ON BRAIN CHOLECYSTOKININ CONCENTRATION IN THE MOUSE. C.A. DeSalvo*, W.A. Bauman*, H. Sershen*, A. Hashim* (SPON: G. Lehrer). Veterans Administration Medical Center, Bronx NY and Center for Neurochemistry, Ward's Island, NY.

Cholecystokinin octapeptide (CCK-8) and dopamine (DA) have been shown to colocalize in meso-limbic neurons. To determine the effect of MPTP (DA neurotoxin) on brain CCK-8 and DA concentrations, Balb/cBY mice were subcutaneously injected with MPTP (30 mg/kg at time 0 and 6 hrs) or vehicle, then on day 5 sacrificed. Striatum (S) and olfactory tubercle (OT) were dissected and stored at -50°C until extraction and assay (radioimmunoassay for CCK-8 and HPLC with electrochemical detection for DA). Results (mean±SEM):

	Section	CCK-8	DA	DOPAC+HVA/DA
Control	OT	1.2±0.1	117± 2‡	0.16±.01
MPTP	OT	1.2±0.1	38±14‡	0.24±.06°
Control	S	1.0±0.1	153±16‡	0.17±.01°
MPTP	S	0.9±0.1	10± 2‡	0.87±.02°

All concentrations: ng/mg protein. HVA=homovanylic acid, DOPAC=dihydroxyphenylacetic acid. $^{\ddagger}p<0.02$, $^{\ddagger}p<0.01$, $^{\circ}p<.001$

Conclusions: (1) MPTP markedly reduced DA concentration in S and OT (2) increased DOPAC+HVA/DA in S suggests a compensatory up regulation of surviving DA terminals (3) MPTP does not decrease CCK-8 concentration in S and OT, possibly because of neuronal differences in uptake and/or metabolism of this neurotoxin.

491.14

IN VIVO DOPAMINERGIC NEUROTOXICITY OF MPP^+ ANALOGS STUDIED BY MICRODIALYSIS OF DOPAMINE AND LACTATE IN RAT STRIATUM. H.Rollema*[1], R.G.Booth* and N.Castagnoli Jr.* (SPON: R.B.Mailman). Division of Toxicology, School of Pharmacy, University of California, San Francisco, CA 94143 and Dept. of Medicinal Chemistry, School of Pharmacy, State University Groningen, 9713 AW The Netherlands[1].

In vivo microdialysis in rat striatum has previously been used to study the effects of MPP^+ on DA-ergic nerve terminals. Changes in the dialysate levels of DA and metabolites indicate the profound and irreversible effects of a 10 min intrastriatal perfusion with 10 mM MPP^+ (Rollema, H. et al., Eur. J. Pharmacol. 126: 345, 1986), while the simultaneous increase in striatal lactate formation reflects the inhibition of mitochondrial respiration by MPP^+ (Rollema, H. et al., J.Pharm.Exp.Therap. in press, 1988). Measurement of these parameters allows the assessment of the in vivo neurotoxicity of analogs of MPP^+, which as charged ammonium species do not enter the brain after systemic administration. The study of such a series of compounds, including substituted phenylpyridiniums, alkylpyridiniums, tricyclic ions and potential endogenous compounds such as (iso)quinoliniums and beta-carboliniums, showed that a number of analogs have MPP^+-like neurotoxic effects after intrastriatal administration. The activities of the compounds range from virtually equipotent to 100-fold less potent than MPP^+ itself. From these data structure-activity-relationships can be studied, which will be discussed in view of the current hypotheses on the mechanism of action of MPP^+. (Supported by NIH Grant NS 23066 and a NATO Science Fellowship to H.R.).

NEUROTOXICITY V

492.1

ENHANCED UPTAKE OF A HEAVY METAL (SILVER) AFTER SURGICAL DISRUPTION OF THE BLOOD-BRAIN BARRIER IN RATS AND MONKEYS. G.F. Alheid, and C.L. Haselton*. Dept. Behavioral Medicine and Psychiatry, Univ. of Virginia, Charlottesville 22908

Single, peripheral injections of silver protein, result in a reliable pattern of labeled neurons in the forebrain and brainstem of rats (Rungby and Danscher, Acta Neurol. Pathol. 60:92,'83), and in the hypothalamus and brainstem of monkeys (Alheid, Anat. Rec. 220:8A,'88), that may be visualized with physical development. In the course of experiments in which animals injected with peripheral silver were subsequently injected intracranially with neuroanatomical tract-tracers, an unusual pattern of neuronal labeling was observed, suggesting that blood-brain barrier disruption incidental to tracer injection resulted in neuronal accumulation of silver, either directly, or possibly after retrograde transport from the area of damaged parenchyma. In the rat, silver labeling of cells (not normally labeled by peripheral injections) included the ventral anterior thalamus, while in the monkey (marmoset) neurons were labeled in substantia innominata, dorsal raphe, and locus coeruleus. In silver injected, but unoperated monkeys, these areas do not contain labeled neurons. In both the monkey and rat dense silver labeling was observed in the cortex at the site of damage incidental to the tracer injections, while in rats and monkeys which received tracer injections but no prior peripheral silver injection, no silver labeling was observed. We are currently examining material from additional monkeys to determine the generality of these observations and to identify any additional areas with "increased" labeling compared to intact controls, and in rats we are testing the possibility that the topography of uptake by neurons may vary with the particular site of blood-brain barrier disruption. Supported by NINCDS 17743 and the Virginia Center on Aging.

492.2

EARLY ESTABLISHMENT OF CHARACTERISTIC METABOLIC ABNORMALITIES AFTER PORTACAVAL SHUNTING. A.M. Mans, M.R. DeJoseph*, D.W. Davis, J.R. Vina*, and R.A. Hawkins. Dept. Anesthesia, Milton S. Hershey Medical Center, Hershey, PA 17033

Portacaval shunting in rats causes many changes in concentrations of brain and plasma metabolites, as well as in glucose use, blood-brain barrier transport, liver-to-body weight ratio and behavior. However, the etiology of the abnormal brain function remains obscure. The time course of these abnormalities after shunting is largely unknown; such knowledge may show correlations between the development of encephalopathy and biochemical alterations.

We have measured the following variables at six time points between 1 day and 60 days after portacaval shunting or sham-operation: brain content of neurotransmitters, amino acids, glucose and ammonia; plasma concentrations of amino acids, glucose, ammonia and lactate; indices of brain glucose use and tryptophan transport across the blood-brain barrier; liver and body weight. All these variables were measured in the same rats. The results showed that many of the changes that occurred after shunting were already apparent as early as 1 day after surgery and nearly all were present after 2 days. With some exceptions the new values were maintained relatively constant throughout the period studied, and therefore had apparently reached new steady-state levels.

Supported in part by NIH Grant NS 16389.

492.3

CEREBELLAR BLOOD-BRAIN BARRIER (BBB) IS DAMAGED BY INGESTED PROTEIN IN BILE DUCT-LIGATED RATS. J.Neiman[1]*, P.A.Stewart,[2] C.R.Farrell[2]*, P.L.Carlen[1,3], and H.Orrego[1,3]*. Addiction Research Foundation[1], 33 Russell St., Toronto, Ontario, and Departments of Anatomy[2] and Medicine[3], University of Toronto., Toronto, Ontario, Canada, M5S 1A8.

Cerebellar damage and liver disease are frequently seen in alcoholics. Furthermore, patients with hepatic encephalopathy can become comatose after ingesting high levels of protein. The pathogenesis of these effects probably includes changes in the composition of the brain extracellular fluid. We questioned whether blood-brain barrier breakdown plays a role in these phenomena. Hepatic cirrhosis was produced in rats by ligating the common bile duct. After 3-4 weeks, half of the experimental and half of the sham-operated rats were force-fed 1.5 g/kg casein hydrolysate. Two hours after feeding the operated rats were stuporous, but unfed operated and all sham-operated rats appeared normal. The integrity of the BBB was evaluated using an intracardiac injection of horseradish peroxidase (HRP). 30 seconds after injection, the rats were decapitated and the brains removed and examined for HRP leakage. Within the cerebral cortex the BBB appeared to be intact in all animals, however in animals with both bile duct ligation and casein feeding, multiple, large leakage spots were found in the cerebellum, primarily in white matter. The BBB damage in the cerebellum suggests that liver disease and dietary proteins may play a role in the cerebellar damage seen in alcoholic patients.

492.4

DOXORUBICIN MYOTOXICITY AND NEUROTOXICITY: INJECTION INTO THE ORBICULARIS OCULI. L.K. McLoon and J. Wirtschafter. Dept. of Ophthalmology, University of Minnesota, Minneapolis, MN 55455.

Doxorubicin is a fluorescent retrogradely transported neurotoxin that has the capability of chemodenervation and chemomyectomy. The effect of injection of doxorubicin on the orbicularis oculi and its motor neurons was examined in monkeys and rabbits. Two mg of doxorubicin in sterile saline was injected into the right lower lids of two cynomolgus monkeys and nine rabbits. The animals were sacrificed four or 64 days after doxorubicin injection. Skin ulceration and necrosis occurred in some of the experimental animals, and when present was completely healed in two to three weeks. The injected muscles showed extensive evidence of degeneration at the injection site with cytoplasmic vacuolization, edema and myofibrillar disorganization. There was little evidence of myotoxic effects in portions of the muscle distant from the injection site. By 64 days after injection, the orbicularis oculi was greatly reduced in muscle mass, with few muscle fibers remaining at the injection site. Counts of the facial motor neurons 64 days after doxorubicin injection indicated there was only a 15% loss of neurons on the injected side. The neurons on the injected side appeared to have a decrease in cell body area, although this was not quantified. Thus, doxorubicin appeared to have a more myotoxic than neurotoxic effect when injected into this muscle system. Its clinical application in the treatment of various muscle spasm diseases will be discussed.

492.5

DIFFERENTIAL EFFECTS OF BUTHIONINE SULFOXIMINE ON GLUTATHIONE LEVELS IN NEUROCHEMICALLY DEFINED REGIONS OF THE MOUSE BRAIN. N. Harary and T.H. McNeill. Dept. of Neurology, University of Rochester, Rochester, NY 14642.

Glutathione (GSH) plays an important role in the protective mechanisms of cells. This study evaluated the effects of butathione sulfoximine (BSO), an inhibitor of GSH synthesis, on GSH levels in the striatum, substantia nigra and locus coeruleus in young and aged mice. All tissues were analyzed using the HPLC system. GSH values in the striatum, substantia nigra and locus coeruleus of the young control animal were 2.14, 1.47 and 1.32 mmol/kg respectively. BSO induced a dose dependent effect on GSH levels in animals injected with 1.5 to 6.0 mM concentrations of the drug. GSH was significantly reduced in the three areas within 5 hours following repetitive injections of 4.5 mM BSO, and remained depressed for 24 hours. However, a return to control values occurred in all tissues after 72 hours. Comparison between young and old controls showed that a 15% decrease occurred in GSH concentrations in the striatum and substantia nigra, while the difference between young and old animals in the locus coeruleus was over 40%. The effect on GSH levels of old mice injected with 3.0 mM BSO was comparable to the effect observed in young animals from the same drug exposure. It is presently unknown whether the further decrement in GSH levels from BSO treatment in aged mice has a critical effect on catecholamine neurotransmitter systems. Supported by PHS grants AG00300, NS16147, Amer. Cancer Soc. and United Parkinson's Foundation.

492.6

AFFERENT FIBER DYSFUNCTION IN VINCRISTINE NEUROPATHY. EFFECT OF GANGLIOSIDE TREATMENT. F. Di Gregorio*, G. Favaro* and M.G. Fiori. Fidia Research Laboratories, 35031 Abano Terme (PD), Italy.

A reproducible model of vincristine (VCR)-induced neuropathy has been obtained by i.v. administration of 0.25 mg/kg VCR in rabbits, once a week for 5 consecutive weeks. Morphological evidence of axon degeneration was accompanied by reduction of sciatic nerve compound action potential (CAP) amplitude, area and conduction velocity (CV). Extracellular recording of single fiber action potential from teased ventral and dorsal roots demonstrated a generalized decrease of CV, involving α and γ motoneurons as well as primary and secondary afferent fibers. Ganglioside (GA) treatment (50 mg/kg/day, i.v.) during the period of VCR administration limited CAP amplitude and area decrease, and corrected single fiber CV pattern. In particular, the percentage of sensory fibers conducting at high speed (>64 m/s) was larger in GA-treated (26%) than in saline-treated animals (16%). The position sensitivity (i.e. rate of firing as a function of muscle stretch) of both primary and secondary afferents from muscle spindles was reduced by VCR administration. In addition, many fibers required an abnormally high degree of stretching to initiate firing. The possibility that GA treatment could protect spindle function, in addition to impulse conduction along sensory fibers, is currently under investigation.

492.7

AXOPLASMIC PROTEIN TRANSPORT DEFICIENCES FOLLOWING CHRONIC EXPOSURE TO ACRYLAMIDE (ACR) AND 2,5-HEXANEDIONE (HD). D.W. Sickles* (SPON: G. Standage) Department of Anatomy, Medical College of Georgia, Augusta GA. 30912-2000

Single injections of acrylamide (ACR) and 2,5-hexanedione (HD) decrease the rate and quantity of protein transport in rat sciatic nerve (Sickles, Toxicologist 8: 43, 1988). These studies (segmental analysis of sciatic nerve radioactivity after 3H-leucine DRG injection) have been extended to determine the overall effect of the neuropathy-producing intoxication schedule upon protein supply to the axon. Over the 24 hour period following a single injection of 50 mg/kg ACR and 4mmoles/kg HD, the rate was equally reduced 6.1% and 8.7%, respectively. Multiple injections (2-10) of ACR or HD produced a flat 7.8% and 13.1% reduction, respectively. The quantity of protein transported was decreased 45% following 1 injection of 50 mg/kg ACR and slowly returned to, but never attained, control levels (-13% at 24 hours). HD (4mmoles/kg) produced a biphasic decrease in capacity of transport. At 1, 16 and 20 hours, a 36, 32 and 33% reduction was observed, but at 8 hours and 24 hours only 9.4 and 2.9% reductions were observed. Two to ten exposures to ACR and HD produced reductions of 49-56% and 51-61%, respectively. Therefore, each toxicant caused a total deficit in protein delivery of 28-30% over the ten day exposure schedule. (Support by NIH OH02020 and MCGRI)

492.8

TOXICOKINETICS OF LINDANE, PICROTOXIN AND Ro5-4864 COMPARED. **D.E. Woolley and H.L. Drummer. Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616.**

The in vivo effects of lindane (5, 10 and 20 mg/kg), Ro5-4864 (5, 10 and 20 mg/kg) and picrotoxin (PTX; 0.5, 1 and 2 mg/kg) administered i.p. in DMSO (0.5 ml/kg) in the rat were compared because of in vitro evidence that each binds to the picrotoxinin site of the $GABA_A$-activated chloride channel. All 3 drugs produced similar types of seizure activity, hypothermia with peak effect at 1 hr, and hypophagia. However, PTX was the most potent of the three in producing seizures and hypothermia and the least effective in reducing food intake. The time course of seizures, hypothermia and hypophagia produced by the same doses of lindane and Ro5-4864 was strikingly similar. However, Ro5-4864 was more effective in producing hypothermia at the highest dose and less effective at the lowest dose. Male rats were more sensitive than females to the effects, including lethal effects, of lindane, whereas the reverse was true for PTX. (The effects of Ro5-4864 were not compared in both males and females.) Thus, though the 3 drugs shared a similar profile of effects in vivo, some differences in the time course and relative effectiveness on the various measurements could be demonstrated.

492.9

DIFFERENTIAL SUSCEPTIBILITY OF SHORT- AND LONG-SLEEP MICE TO BRAIN WEIGHT DEFICITS FOLLOWING PRENATAL ALCOHOL EXPOSURE. C.R. Goodlett, D. M. Gilliam, J.M. Nichols and J.R. West, University of Iowa, Iowa City, IA 52242.

The long-sleep (LS) and short-sleep (SS) lines of mice have been selectively bred for high and low sensitivity as adults to the hypnotic effects of alcohol. The two lines may also differ during development to the neurotoxic effects of prenatal alcohol exposure. Evidence from behavioral studies following prenatal alcohol exposure suggests that LS mice are more susceptible to developmental behavioral deficits than SS mice. The present study examined brain size in adult mice of the two lines given gestational treatment with alcohol. LS and SS dams were treated from gestational days 7-18 with alcohol (either 3.0, 4.0 or 4.5 g/kg twice a day), with an isocaloric control solution (sucrose or maltose-dextrin), or served as unintubated controls. For the LS offspring, there was significant, dose-dependent reduction of brain and body size in adults prenatally exposed to alcohol. For the SS offspring, prenatal alcohol exposure had no significant effects on adult brain and body size. Interestingly, the use of sugar solutions as controls resulted in significant reductions in brain size in the SS (but not LS) lines, compared to unintubated controls. The severity of fetal alcohol effects on the developing CNS may depend in part on genetically determined differences influencing sensitivity to alcohol of the adult CNS. (Supported by NIAAA grant #AA07313 to J.R.W. and #AA06939 to DMG)

492.10

IN VITRO NEUROTOXICITY OF METHYL IODIDE AND METABOLITES: GLUTATHIONE ATTENUATION. C.J. Davenport, D.A. Neptun* and K.T. Morgan*. Experimental Pathology and Toxicology, CIIT, Research Triangle Park, NC 27709.

Following oral administration, methyl iodide (MeI;a human neurotoxin) is metabolized to S-methylglutathione and further hydrolyzed to S-methyl-cysteine, glutamate (GM) and glycine (Johnson, J. Biochem.,98:30,1966). The purpose of the present work was to determine whether MeI neurotoxicity could be induced in vitro by the parent compound or selected metabolites, and whether addition of glutathione inhibited neurotoxicity. Mature (15-21 days in vitro) dissociated murine (CD-1 strain) neocortical cultures were exposed to a range of MeI concentrations (0.1-100mM) for 5 min or 1 day; cytotoxicity was assayed 24-36 hr later. Cell death was dependent upon exposure concentration and duration, with a neuronal ED50 of 5mM and <1mM MeI for 5 min and 24 hr exposures, respectively. Although GM was the only metabolic product to induce neuronal injury, MeI toxicity was not believed to be GM-mediated since: (a) The neuronal ED50 for a 5 min exposure to GM is 50-100μM (Choi et al., J. Neurosci.,7:357,1987), (b) The glutamate antagonist DL-2-amino-5-phosphonovaleric acid did not protect against MeI induced injury and (c) Cytotoxicity to MeI but not to GM was reduced by simultaneous addition of reduced glutathione. These results indicate that MeI neurotoxicity probably results from interaction of the parent compound (or unknown metabolites) with protein sulfhydryl groups in brain tissue.

492.11

TYPE II BUT NOT TYPE I PYRETHROIDS ALTER AMYGDALOID KINDLING IN THE RAT. ME Gilbert, CM Mack*, S Acheson*. Northrop Environmental Sciences, RTP, NC 27709.

Type I and Type II pyrethroids both increase sodium channel conductance; Type I's (e.g. cismethrin, CSM) leading to enhanced nerve excitability and repetitive firing, Type IIs (e.g. deltamethrin, DLT) producing nerve inexcitability via a depolarization block. Type II pyrethroids have also been reported to decrease inhibition by antagonism of GABA function. The functional impact of pyrethroids on the CNS may depend on the relative contribution of these contrasting actions. Electrical kindling of the amygdala in rats was assessed in an attempt to dissociate the net effect of these mixed actions. CSM (15 mg/kg, po) or DLT (6 and 10 mg/kg, po) were administered 2 hours prior to daily kindling stimulation (60 Hz, 1-s train biphasic squarewaves at 200 μa) and the rate of development of generalized seizures was compared with controls receiving the corn oil vehicle. DLT (10 mg/kg) facilitated the development of kindled seizures. Additionally, in the absence of stimulation, 3 of 14 animals were observed to experience spontaneous seizures following the 3rd-5th administration of DLT. Subjects treated with CSM did not differ from controls. These data stand in contrast to a previous report in which both Type I and II pyrethroids decreased CD-50 for PTZ (Devaud et al, 1986). DLT may enhance amygdaloid kindling through an antagonism of GABA-mediated inhibition.

492.12

SUPEROXIDE DISMUTASE ACTIVITY IN BRAIN BY STEADY-STATE KINETIC ANALYSIS. Jayasimha Narayanamurthy* & S.P. Mahadik, Div. Neuroscience, NYS Psychiatric Inst., Depts. of Psychiatry, and Biochemistry & Molecular Biophysics, Physicians & Surgeons, Columbia U., New York.

Superoxide dismutase (SOD) levels in CNS are critical in protecting neural function from free radical toxicity after CNS injury (mechanical, ischemic, neurotoxic or metabolic). SOD is often assayed by procedures which involve enzymatic or non-enzymatic generation of superoxide radicals (O_2^-) and their interaction with the suitable detector. We report a procedure in which non-enzymatic generation of steady-state levels of O_2^- is achieved by the interaction of phenazine methosulfate (PMS) with NADH generated by the alcohol dehydrogenase system. The O_2^- generation was monitored (540nm) as nitroblue formazan formation. The reaction was linear over 5min and showed a stoichiometric relationship with NADH production, O_2^- generation and NBT reduction. Under these conditions saturation level of O_2^- was maintained with 1-6U of SOD (bovine erythrocyte) and used for calculation. This rapid & reproducible method determines 0.1U of SOD (2mg wet wt of tissue) using a linear rate of reaction. SOD was determined in extracts (0.1M PO4 buffer, pH 7.8) obtained from different anatomical regions of rat brain. The levels correlated with the regional catecholamine turnover consistent with the possible free radical formation during neuronal activity.

492.13

TIME COURSE OF QUINOLINIC ACID-INDUCED NADPH-DIAPHORASE-CONTAINING NEURON CELL DEATH IN RAT STRIATUM. C.M. Wray* and R.J. Boegman (SPON: C. Romero-Sierra). Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Previous studies have shown that intrastriatal injections of excitotoxins destroy NADPH-diaphorase-containing neurons. We sought to establish the time course and dose-response relationship of neuronal cell death following intrastriatal injections of quinolinic acid (QUIN). Diaphorase-positive neurons appeared swollen and sometimes showed markedly varicosed dendrites three and six hours after an intrastriatal injection (QUIN, 120 nmol/μl). At nine hours, a few swollen cells without dendrites were observed, and by 12 hours post injection, a full lesion had developed with no diaphorase-positive neurons in the core injection area. A dose-response relationship was established by injecting different doses of QUIN into the striatum and determining the number of diaphorase-positive neurons. Injections of 2.5 nmol/μl resulted in a diaphorase cell survival rate of 78.6%, while with 7.5 nmol/μl only 13% remained diaphorase-positive. Our results clearly indicate that in the core injection area which most closely represents the area of the striatum exposed to the injected dose of excitotoxin, NADPH-diaphorase-containing neurons are very sensitive to the neurotoxic action of QUIN.
(Supported by the Medical Research Council of Canad)

492.14

QUINOLINIC ACID STRIATAL LESIONS DIFFERENTIALLY AFFECT MALE AND FEMALE RATS. E.M. Zubrycki, A.B. Norman, M.T. Shipley, and P.R. Sanberg. Laboratory of Behavioral Neuroscience, Depts. of Psychiatry, Psychololgy, Physiology, Anatomy, and Neurosurgery, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Excitotoxic lesions of the striatum in male rats result in temporary marked weight loss resembling the lateral hypothalamic syndrome (1). Conversely, it was reported that striatal kainic acid lesions in female rats led to increases in weight and consummatory behavior and further, that decreases in dopamine density were predictive of the resulting weight gain (2). The present study utilized the excitotoxin, quinolinic acid, to lesion the intrinsic neurons of the striatum of male, female, and ovariectomized rats.

Sprague-Dawley rats (n=41) received bilateral injections of 150 nml quinolinic acid or vehicle (1 ul). Body weights were monitored daily and tabulated as percent of original weight. It was found that male rats suffered more dramatic and extended weight losses as compared to females. Although females recovered more rapidly, they did not demonstrate increased weight gain as compared to controls. Ovariectomized rats showed a pattern of weight loss that was a compromise between male and female, but did not differ from either group. Weight gain observed in control groups did not significantly differ.

These results confirm a differential response to excitotoxin lesions between sexes.

1. Sanberg & Fibiger, Exp. Neurol. 66:444, 1979.
2. Deckel et al., Abst. of East. Psychol. Assoc. 57:72, 1986.

492.15

POSSIBLE MECHANISM FOR NEUROTOXICITY OF FLUOROACETATE AND FLUOROCITRATE. C. S. Hornfeldt, A. A. Larson. Department of Veterinary Biology, University of Minnesota, St. Paul, MN, 55108.

The mechanism by which fluoroacetate (FA) and fluorocitrate (FC) exert their neurotoxic effects is unknown. It is known that FA is metabolized to FC, a Krebs Cycle inhibitor which causes the accumulation of citrate. Chelation of calcium by accumulated citrate in FA-poisoned animals has been proposed to be responsible for some of the peripheral effects of FA toxicity. To examine the effects of FA and FC in the CNS, and determine whether chelation of calcium is responsible for the convulsant effects of FA, we injected FC intrathecally (IT) in mice. Seizures occurred almost immediately after either FA or FC at the upper end of the dose-response curve. Intracerebroventricular injection of FC also produced seizures but required higher doses and occurred only after a latent period of greater than 40 min. This confirms previous work in the cat and rat suggesting that the spinal cord is the site of action of FA- and FC-induced seizures. While it has been previously demonstrated that cat spinal cord is 300-400 times more sensitive to FC than FA, we found FC to be 2500 times more toxic than FA when injected IT in mice. To determine whether chelation of cations is capable of producing similar actions to those observed after FA and FC, we injected mice IT with citrate, EGTA and EDTA. Each agent produced behavioral effects similar to those seen after FA and FC, both in onset and in molar potency. Coadministration of IT calcium or magnesium with FC or EDTA delayed the onset of seizures after FC and increased the dose of EDTA required to elicit seizures. These results suggest that these agents exert their CNS toxicity by decreasing the concentration of ionized cations in the spinal area. (Supported by Grants DA04090, DA00124 and DA04190)

492.16

DEPTH OF CHLORAL HYDRATE ANESTHESIA AS A FUNCTION OF PIGMENTATION AND STRAIN DIFFERENCES IN RATS. D.F. Sisson* and J. Siegel. School of Life and Health Sciences, Univ. Delaware, Newark, DE 19716 and I.S. Westenberg. Glendale College, Glendale, AZ 85302.

In a previous study we used EEG power spectrum analysis as an indicator of depth of chloral hydrate anesthesia and as a predictor of changes of visual evoked potentials (VEPs) in Wistar (W) albino rats. We are now concerned with the generalizability of our findings. The current report investigates differences in power spectra, depth of anesthesia and VEPs as a function of albinism vs. pigmentation and strain differences. Five Long-Evans (LE) rats, 3 pigmented and 2 albino, were compared to each other and to 12 albino W rats. Using EEG power spectral analysis as an indicator of anesthetic depth, it was found that W albino rats are much more sensitive to chloral hydrate anesthetic: 30-40 mg i.v. produced a deep level of anesthesia for 30 min and a moderate level for about 30 min more. In contrast, all LE rats were less sensitive to chloral hydrate: both pigmented and albino rats went deep for only 5-10 min with a 40-90 mg i.v. dose and the subsequent moderate state lasted only 20-25 min. VEPs in both strains were about the same. We conclude that strain difference (W vs. LE) is important in determining sensitivity to chloral hydrate anesthesia and albinism vs. pigmentation is not relevant. This work was supported in part by ARO Contracts DAAG 2980K0015 and DAAL 0388K0043.

492.17

EARLY ACTIVITY DECREMENTS IN GROUPED AND ISOLATED MICE AFTER EXPOSURE TO IONIZING RADIATION. H.D. Davis*, D.M. Maier*, and M.R. Landauer* (SPON: B.M. Rabin). Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

The effect of gamma photon irradiation on locomotor activity was examined in male Swiss-Webster mice that had been pre-housed individually or in groups of 10 for 4 wk prior to radiation exposure. Mice in each pre-housing condition received 10 Gy cobalt-60 radiation (1 Gy/min) or a sham irradiation procedure, forming 4 groups (isolated-sham, grouped-sham, isolated-irradiated, and grouped-irradiated) (N=12/group). Locomotor activity (ambulation, rearing) was monitored individually for 12 hr postirradiation (PR). Radiation produced significant activity decrements from 45 min to 8 hr PR in both grouped and isolated animals. At 4-6 hr PR, activity in the irradiated groups was only 25% of sham irradiated groups (the largest decrement observed). The percent decrease in the activity of irradiated animals (compared to control groups) was similar in both grouped and isolated mice, however, the absolute amount of the activity decrement produced by radiation was smaller in grouped mice than isolated mice due to the lower activity of the grouped control subjects (40-60% of isolated controls 2-7 hr PR).

ALZHEIMER'S DISEASE: TRANSMITTERS

493.1

CEREBRAL CORTICAL CHOLINERGIC REDUCTION IN DOMINANTLY-INHERITED OLIVOPONTOCEREBELLAR ATROPHY (OPCA): IMPLICATIONS FOR THE CHOLINERGIC HYPOTHESIS OF DEMENTIA. S.Kish*, Y.Robitaille*, M.Freedman*, M.El-Awar*, L.Schut*, L.Chang*, and M.Rebbetoy*. (SPON: R. Harrison). Human Brain Lab, Clarke Inst. Psychiat., Toronto, Ontario, Canada M5T 1R8.

Much circumstantial evidence suggests that a brain cholinergic reduction may underlie the dementia of Alzheimer's disease (AD). We measured the behaviour of the cholinergic marker enzyme cholineacetyltransferase (ChAT) throughout the brain of four patients from one OPCA family. ChAT activities were markedly reduced (-50 to -80%) in all (n=27) examined cerebral cortical subdivisions. However, in contrast with observations in AD brain, ChAT levels in amygdala and hippocampus were generally normal or only mildly reduced. Neuropathological analysis revealed a severe loss (-70%) of cholinergic cell bodies throughout the nucleus basalis but with an absence of senile plaques and tangles. Since our OPCA patients had only mild cognitive impairment we conclude that a severe cortical cholinergic loss alone is not sufficient to produce the cognitive deterioration of advanced AD. OPCA may represent a non-AD patient group having a distinctly different pattern of brain cholinergic disturbance which may be useful for studies of behavioural consequences of a more selective cholinergic lesion. (Sup. by MRC of Canada).

493.2

REDUCTION IN SOMATOSTATIN AND CHOLINE ACETYLTRANSFERASE IN RAT BRAIN FOLLOWING QUINOLINIC ACID ADMINISTRATION INTO THE LATERAL VENTRICLE K. Maeda, S. Kuo*, H. Kaneda*, T.N. Chase, C.A. Tamminga NINCDS, NIH, Bethesda, MD 20892, Maryland Psychiatric Research Center, University of Maryland, Balto., MD 21228

Recent evidence suggests that certain neurodegenerative diseases like Alzheimer's disease (AD) may be mediated by the neurotoxic action of excitatory amino acids (EAA). In the present study, we have examined the effect of QA administration in rats into the lateral ventricle on SRIF contents and CAT activities. Daily injections of 10 ug QA for 7 days and a continuous infusion of 80 ug in 14 days were the two administration techniques compared. The repeated manual injection of QA for 7 days did not alter either SRIF content or CAT activity in brain. The QA infusion, however, resulted in a significant reduction in CAT activity in the R hippocampus (0.187 $\pm$ 0.020 nmol/min/mg tissues vs 0.301 $\pm$ 0.038, $p<0.05$) and the R striatum (0.955 $\pm$ 0.072 vs 1.238 $\pm$ 0.074, $p<0.05$), compared to the controls. A reduction in SRIF content was obtained in the R anterior cortex (0.391 $\pm$ 0.060 pg/ug prot vs 0.636 $\pm$ 0.070, $p<0.05$) and the R striatum (0.270 $\pm$ 0.030 vs 0.548 $\pm$ 0.050, $p<0.05$). We also observed a reduction in the SRIF content in the right hippocampus, although the difference was not statistically significant ($p<0.06$). SRIF content in the tissues from the left side (non-cannulated) were not significantly affected by the administration of QA. These data suggest that a heuristic animal model of AD could be developed by the technique of continuous QA infusion into the ventricular system.

493.3

ALZHEIMER'S DISEASE: SELECTIVE LOSS OF M1 RECEPTORS IN HIPPOCAMPUS AND INCREASES IN M1 AND M2 RECEPTORS IN STRIATUM AND NUCLEUS BASALIS

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We have employed quantitative autoradiography to examine the pre- and postsynaptic elements of the muscarinic cholinergic system in several regions of postmortem brain tissue from 4 Alzheimer's cases and age-matched controls. The density of M1 receptors ([^{3}H]pirenzepine) was decreased in hippocampus (35%), whereas the density of M2 receptors ([^{3}H]N-methylscopolamine with excess pirenzepine) was not changed. No changes in the density of either subtype were apparent in cortical regions. In striatum both M1 (>100%) and M2 (66%) receptors were significantly increased in density as compared to controls. Patches of very dense binding of M1 and M2 receptors were observed within the nucleus basalis of Meyert in the Alzheimer's cases. The density of choline uptake sites ([3H]hemicholinium-3) was decreased in both hippocampus and striatum, suggesting that a cholinergic deficits in Alzheimer's disease are not restricted to the cholinergic system of the hippocampus. Analysis of dopaminergic and serotoninergic systems is currently being pursued.

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493.4

CHANGES IN NICOTINIC RECEPTORS IN HUMAN AND RAT CNS. E. Giacobini, P. DeSarno,* B. Clark, M. McIlhany. Southern Illinois Univ. Sch. Med., Dept. Pharm., Springfield, IL 62794-9230.

Among the severe deficits seen in the cholinergic system in Alzheimer's disease (AD), a significant decrease in high affinity nicotinic binding sites has been observed. The effect of cholinominetic therapy with cholinesterase inhibitors or cholinergic agonists may be influenced by presynaptic nicotinic receptor function and acetylcholine (ACh) release. We have examined the effect of long- lasting intracerebroventricular (i.c.v) administration of physostigmine (4 ug, twice daily under 13 days) on ^{3}H-ACh release, nicotinic receptor binding and AChE in various brain regions (cortex, striatum and hippocampus) of the rat. We have also compared nicotinic receptor binding in both bioptic and autoptic samples of human frontal cortex of AD patients and controls. Biochemical findings have been correlated to clinical and pathological diagnoses. Nicotinic receptors were characterized by means of three ligands (nicotine, alpha-bungarotoxin and K-bungarotoxin). Comparable decreases in nicotinic binding were observed in both autoptic and bioptic tissue which were closely correlated to clinical and pathological findings. Our studies suggest that frontal cortex biopsy may constitute a useful complement to clinical and pathological examinations in AD. (Supported in part by National Institute of Aging AG05416).

493.5

ALTERATIONS IN NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVE PROFILES IN THE BASAL FOREBRAIN OF ALZHEIMER'S AND PARKINSON'S PATIENTS. E.J. Mufson[1], D.M. Gash[2], M.A. Bothwell[3], L.B. Hersh[4] and J.H. Kordower[2]. [1]Institute For Biogerontology Research, Sun City, AZ 85351, [2]Dept. of Neurobiol. and Anatomy, Univ. Rochester Sch. Med., Rochester, N.Y. 14642, [3]Dept. Psysiol. & Biophysics, Univ. Washington, [4]Dept. Biochem. Univ. Texas Health Sci. Ctr.

Previous studies of basal forebrain (Ch1-Ch4) neuronal dysfunction in Alzheimer's (AD) and Parkinson's (PD) disease have been performed using Nissl or acetylcholinesterase stains. In the present investigation pathology associated with basal forebrain neurons expressing nerve growth factor receptor (NGFR) was evaluated using a monoclonal antibody directed against human NGFR. Brain slabs were obtained from seven AD (68-78 yrs), one demented PD (74 yrs), one non-demented PD (88 yrs) and four neurologically normal (68-78 yrs) cases. Tissue was fixed for 24 hr in 4% paraformaldehyde and processed immunohistochemically for the localization of NGFR singly or dually for choline acetyltransferase (ChAT). AD and PD was confirmed postmortem using Thioflavin-S and Bielschowsky methods or by the demonstration of cell loss and Lewy bodies in the substantia nigra using H&E. Aged control brains revealed a continuum of NGFR immunoreactive neurons extending throughout the basal forebrain (Ch1-Ch4) with an average of 190,000 positive perikarya per hemisphere. NGFR neurons ranged in shape from small oval cells located within the medial septum (Ch1) to multipolar magnocellular perikarya within Ch4. In AD, subsectors of the basal forebrain exhibited neuronal loss, shrinkage, dystrophic fibers as well as normal appearing NGFR neurons. Cell counts revealed the greatest reduction in NGFR positive neurons (48-83%) primarily within Ch4 as compared to Ch2. There was no consistent pattern to the cell loss observed in AD. Within the Ch subfields NGFR and ChAT colocalized indicating that the NGF receptor does not deccuple from cholinergic neurons in AD. NGFR and ChAT stained basal forebrain sections counterstained with Thioflavin-S revealed numerous ghost NFTs as well as positive tangle bearing neurons. In AD, there was a striking reduction in NGFR immunoreactive fibers within the external capsule enroute to cortex. In PD cases, there was a severe loss of nucleus basalis NGFR positive neurons primarily in Ch4p in the non-demented and no loss in the demented patient. There were virtually no neuritic plaques in any cortical region in the PD cases. These findings indicate that NGFR positive basal forebrain cells are severely affected in AD and also in PD.

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493.6

NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY IN THE HUMAN AND MONKEY BRAIN: DISTRIBUTION AND COLOCALIZATION WITH CHOLINERGIC ENZYMES. J.H. Kordower[1], D.M. Gash[1], M.A. Bothwell[2], L.B. Hersh[3], and E.J. Mufson[4]. [1]Dept. of Neurobiology and Anatomy, Univ. Rochester Sch. Med., Rochester N.Y. 14642, [2]Dept. Psysiol & Biophysics, Univ. Washington, [3]Dept. Biochem., Univ. Texas Health Sci. Ctr., [4]Institute For Biogerontology Research, Sun City, AZ 85351.

Brains from four normal aged humans (68-78 yrs) and six Cebus apella monkeys (3-13 yrs) were fixed with 4% paraformaldehyde and processed for the localization of nerve growth factor receptor (NGFR), either alone or in combination with choline acetyltransferase (ChAT), and acetylcholinesterase (AChE) via immuno-histochemical and immunofluorescence procedures. In both species, NGFR containing neurons were found almost exclusively within the basal forebrain (Ch1-4). In the monkey, occasional NGFR positive neurons were also observed in the putamen. Far fewer NGFR immunoreactive neurons were observed within the medial septum (Ch1) of the human, as compared to the monkey. In both species, NGFR positive neurons were seen within the vertical limb of the diagonal band (Ch2) and a few small fusiform NGFR-immunoreactive neurons corresponding to the horizontal limb of the diagonal band (Ch3) were observed near the base of the brain. NGFR-immunoreactive magnocellular and parvicellar neurons were observed throughout the nucleus basalis (Ch4) including the anteromedial, anterolateral, intermediodorsal, intermedioventral and posterior subdivisions. NGFR fibers from these neurons coursed within the external capsule and temporal limb of the anterior commissure to reach the cortex and amygdala. Scattered NGFR positive fibers were also seen in the putamen. While many NGFR containing neurons were observed within the medullary laminae of the globus pallidus and within the internal capsule in monkey, far fewer were seen in these regions in human brain. In both monkey and human, numerous NGFR containing neurons were observed within the bed nucleus of the stria terminalis and a few were seen lodged within the NGFR fibers of the dorsal columns of the fornix. In all of these regions, NGFR colocalized with ChAT or AChE (<95%) in both species. Except for NGFR immunoreactive neurons within the monkey mesencephalic nucleus of the trigeminal nerve, NGFR-immunoreactivity was not observed in other brain regions including Ch5 and Ch6. These findings demonstrate that the NFG receptor is an excellent marker for cholinergic basal forebrain neurons in primates making it a valuable tool for assessing the integrity of the primate basal forebrain in age, disease, or experimental manipulation.

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493.7

NEUROPLASTIC POTENTIAL OF CHOLINERGIC NEURONS THAT PROJECT TO THE BASOLATERAL AMYGDALA. G.R. Stewart, M.E.Mueller*, M.T. Price and J.W. Olney. Dept. of Psychiatry, Washington University Sch. of Med., St. Louis, MO 63110.

Degeneration of the basal forebrain cholinergic (BFC) system in Alzheimer's disease (AD) does not appear to entail any recovery either because this system lacks such potential, or the disease process overcomes this potential. Here we have studied the rat basolateral amygdala (BLA) to determine whether cholinergic reinnervation occurs in this nucleus after excitotoxic destruction of BFC neurons that project to it. Adult male rats received unilateral injections of the excitotoxin N-methyl-aspartic acid (50 nmol in 0.4 µl) into the ventral pallidum. Following survival times of 10, 30, 60, 120 or 240 days, animals were either decapitated and their brains frozen for biochemical measurements or were transcardially perfused with an aldehyde fixative and the brains sectioned (50 µm, coronal) on a Microslicer and stained for acetylcholinesterase-histochemistry (AchE-HC). Lesions were well confined to a portion of the ventral pallidum which, from retrograde tracer studies, is known to contain most of the BFC neurons that project to the BLA. The status of cholinergic innervation of the BLA was evaluated by AchE and cholineacetyltransferase assay in microdissected samples of BLA and by AchE-HC videodensitometry which we have found to be a reliable method for monitoring cholinergic innervation of BLA. At 10 days post-lesion, there was a 50% loss of staining/enzyme activity (ipsi versus contralateral BLA compared) which persisted through the 30 and 60 day interval then diminished to 42 and 35% loss at 120 and 240 days respectively. Thus, at 4 and 8 months post-lesion there was evidence for gradual cholinergic reinnervation of BLA . Therefore, this system may have some reinnervation potential, but in the context of AD it would probably be insufficient to compensate for the progressive loss of BFC neurons that occurs as a function of the disease process. Supported in part by Research Scientist Award MH 38894 (JWO), AG 05681 and The Josiah Macy Jr., Foundation.

493.8

GALANIN HYPERINNERVATES SURVIVING NEURONS OF THE HUMAN BASAL NUCLEUS OF MEYNERT IN DEMENTIAS OF ALZHEIMER'S AND PARKINSON'S DISEASE. V. Chan-Palay, The Neurology Clinic, University Hospital, CH-8091 Zürich, Switzerland.

The aim of this study is to find the similarities and differences in Galanin innervation of the cholinergic basal nucleus neurons in these dementing disorders as compared to the controls. Immunocytochemistry with antibodies against galanin peptide and against choline-acetyltransferase is applied on perfused brain preparations. Galanin peptide is present in the basal nucleus of Meynert neuron networks in the normal human brain; in local circuit neurons and a number of galanin/cholinergic neurons. In SDAT, there is a loss of cholinergic neurons. Galanin networks demonstrate an inverse relationship to the cholinergic cell loss. Galanin axons hypertrophy and hyperinnervate the remaining cholinergic neurons. In Parkinson's disease the loss of cholinergic neurons accentuated in dementia and the hypertrophy of the galanin axonal networks on cholinergic neurons is dramatic in Parkinson's with dementia and exuberant in the atypical cases of dementia non-responsive to L-Dopa. These findings suggest that the present therapy of cholinergic enhancement as a means to retard intellectual deterioration can have little effect. The activity of galanin peptide needs to be reduced, e.g. by galanin peptide antagonists or small organic molecules that interact with the galanin receptors.

493.9

EVIDENCE FOR TRANS-SYNAPTIC DEGENERATION OF CHOLINERGIC AFFERENTS TO THE LOCUS CERULEUS IN ALZHEIMER'S BRAIN. R. Strong,* J.S. Huang,* S.S. Huang,* H.D. Chung,* C. Hale,* M.A. Moore* and W.J. Burke* (SPON: F.A. Mithen). Ger. Res. Ed. and Clin. Ctr., VA Med. Ctr.; St. Louis Univ. Sch. of Med., St. Louis, MO 63125.

Increasing evidence suggests that much of the subcortical pathology of Alzheimer's disease (AD) may be due to trans-synaptic retrograde degeneration of neurons projecting to affected cortical areas. The same may be true of neurons projecting to affected subcortical nuclei. We measured neuron density in the locus ceruleus (LC) of autopsied brains of neurologically normal individuals and AD patients. Neuron density in the LC of AD cases was significantly reduced to approximately 50%. We assayed choline acetyltransferase (ChAT) activity as a marker of cholinergic LC afferents. ChAT activity was reduced by about 50% in AD cases. Significantly, the loss of LC neurons was highly correlated with loss of presynaptic ChAT activity. We measured the effect of LC extracts on mitogen activity in 3T3 cells as a nonspecific measure of trophic factors. Mitogen activity was significantly reduced (50%) in the AD group. Mitogen activity was significantly correlated with ChAT activity and the density of neurons in the LC in all cases. These data are consistent with the hypothesis that trans-synaptic retrograde degeneration of cholinergic projections to the LC occurs in AD.

493.10

TETRAHYDROBIOPTERIN AND BIOGENIC AMINE METABOLISM IN ALZHEIMER'S AND OTHER NEUROPSYCHIATRIC DISEASES. R.A. Levine, P.A. LeWitt*[1], N. Pamara*, D. Gurevich*, and M. Stanley, Lafayette Clinic and Wayne State Univ. Sch. of Med., Detroit, MI 48207, and [1]Sinai Hospital of Detroit, Detroit, MI.

Tetrahydrobiopterin (BH_4), as cofactor for phenylalanine, tyrosine, and tryptophan hydroxylases, plays an important role in regulating the synthesis of dopamine and serotonin in the central nervous system, and altered BH_4 metabolism has been associated with certain neurological disorders. In BH_4 biosynthesis, GTP cyclohydrolase (CH) converts GTP to dihydroneopterin triphosphate (NH_2TP); NH_2TP is converted to 6-pyruvoyl-tetrahydropterin (6-PPH_4) by 6-PPH_4 synthase; 6-PPH_4 is converted by one or two enzymes to BH_4, the last enzyme being sepiapterin reductase (SR). NH_2 and neopterin appear only in primate fluids due to intracellular dephosphorylation of NH_2TP.

We have measured the content of BH_4 and neopterin in CSF from a variety of neuropsychiatric patients. In Alzheimer's disease, BH_4 content was decreased by 40%, suggesting that altered BH_4 metabolism may be involved in Alzheimer's disease. CSF neopterin levels were unchanged. BH_4 was well correlated with both homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in CSF, which supports the importance of BH_4 as a regulator of biogenic amine synthesis. Studies are underway to measure certain markers of BH_4 metabolism in various areas of Alzheimer brain tissue.

493.11

CORTICAL β-ADRENERGIC BINDING SITES INCREASE IN ALZHEIMER'S DISEASE. P.J. Whitehouse, M. Tabaton*, R.N. Kalaria and J.R. Unnerstall. Departments of Neurology and Pathology, Case Western Reserve University Sch/Med, Cleveland, OH 44106.

In a sample of six Alzheimer Disease (AD) cases and six age-matched controls, we have determined the extent of neuronal pathology in the locus coeruleus (LC) and compared differences between diseased and control tissues with regard to pathology and changes in β-adrenergic binding sites in the frontal cortex. In the AD cases, neuronal density in the LC was reduced by 30-50%. In the frontal cortex, a 20-30% decrease in neuronal density was observed. Using receptor autoradiographic techniques, we found significant increases in the density of cortical β-adrenergic binding sites in the AD cases. Using drugs that selectively mask either the β_1- or β_2-receptor subtypes, significant increases in the density of both β_1- and β_2-adrenergic receptors were seen. The increase in β_1 binding was localized to the deeper cortical lamina, while the increases in β_2 binding were more generally observed throughout the cortex. In a larger sample, these findings were confirmed by homogenate binding. Since these increases in β-adrenergic binding were seen in both homogenate and autoradiographic experiments, and changes in receptor subtype were regionally selective, these changes indicate that the cortex can still exhibit a plastic response to noradrenergic denervation in AD.

493.12

LOCUS COERULEUS CELL LOSS IN ALZHEIMER'S AND PARKINSON'S DISEASE: PATTERN ANALYSIS. K.F. Manaye, W.K. Smith, E. Weidmer-Mikhail*, C.L. White*, D.J. Woodward and D.C. German. Depts. of Psychiat., Physiol., Cell Biol. and Pathol., UT Southwestern Med. Cntr., Dallas, TX 75235.

Several studies indicate that there is extensive loss of locus coeruleus (LC) cells in Alzheimer's (AD) and Parkinson's disease (PD). We wished to test the hypothesis that the pattern of cell loss is different in the two disease conditions. Using computer imaging techniques and neuromelanin as the cell marker, we examined the bilateral distribution of cells in 5 PD, 5 AD and 4 normal brains (mean ages of 69, 76 and 67, respectively). Cell locations from 16 sections, 0.8 mm apart, were entered into a computer. There was a greater total loss of LC cells in PD (64%) than in AD (52%). The rostral-caudal distribution of cell numbers differed in the AD and PD brains with the greatest disparity occurring in the middle to caudal third of the LC. There was no right-left asymmetry in LC cell number in the normals or either disease condition. The 3-dimensional pattern of cell loss will be analyzed further for both diseases. Research supported by the Dallas Area Parkinsonism Society, Biological Humanics Foundation and the ADRDA.

493.13

NORADRENALINE IN POST-MORTEM BRAIN IN DOWN'S SYNDROME AND ALZHEIMER'S DISEASE. H.Karlinsky,* S.Kish,* Y.Robitaille,* L.Becker,* J.Gilbert,* K.Shannak,* O.Hornykiewicz. (SPON: P.Li). Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario, Canada M5T 1R8.

We measured the concentration of the neurotransmitter noradrenaline(NE) in autopsied brain of ten infant children(aged 3 months to 12 years) and four adults(aged 22 to 59 years) with Down's Syndrome(DS). This study was prompted by evidence demonstrating 1) markedly increased risk for the early development in DS of the brain neuropathological and clinical changes of Alzheimer's disease (AD) and 2) reduced levels of NE in AD cerebral cortex and hypothalamus. Brain histological analyses revealed the typical changes of AD(plaques,neurofibrillary tangles) in the three oldest DS individuals(43,55 and 59 years). When compared with age-matched controls, NE levels were markedly reduced(-50 to -80%)in the cerebral cortex of the three oldest DS cases. The magnitude of this reduction was similar to that observed in AD brain. Mean NE levels in young DS cerebral and cerebellar cortex,limbic brain and basal ganglia (aged 3 months to 12 years) were not significantly different from age-matched controls. Our biochemical data suggests that DS individuals do not begin life with an AD-type brain NE reduction, but that such changes are age-dependent.

493.14

CYTOPATHOLOGY OF PEPTIDERGIC NEURONS IN ALZHEIMER'S DISEASE AMYGDALA. P.B. Cipolloni B.J. Quigley Jr.*, and N.W. Kowall. Dept. Anatomy, Boston Univ., ENR Memorial VA Hosp., Bedford MA 01730 and Neurology Service, Mass.General Hospital, Boston MA 02114.

The amygdala often has the highest density of Senile plaques (SP) in Alzheimer's disease (AD) brain but the relationship of SP distribution to defined neurons in the amygdala is not well understood. We used immunoperoxidase methods to examine somatostatin (SS), neuropeptide Y (NPY), cholecystokinin (CCK), and substance P neurons in the amygdala of 6 AD patients and 6 age-matched controls. SP were defined with Thioflavine S and the silver method of S.K. Campbell et al. (Soc Neurosci Abs 13:678). SP are distributed in all nuclei of the amygdala without a clear mediolateral gradient. The ventral region and paralaminar nucleus are relatively spared. SS and NPY neurons are present in the dorsal part of the lateral (L), accessory basal (AB), and lateral central (Cl) nucleus and the periamygdaloid cortex (PAC). Morphologically these neurons are aspiny stellate cells (Class II). In AD perikaryal density is not visibly altered but fiber density is reduced and neurites in SP are seen with much greater frequency in the cortical and superficial AB nucleus. CCK neurons are found in substantial numbers in the AB and PAC where they contribute heavily to SP formation. Substance P neurons are present in AB, Cl, PAC and dorsal basal magnocellular nucleus where they infrequently contribute to SP. Despite the occurrence of SP throughout the amygdala only SP in medial regions contain peptidergic fibers. This differential incorporation of defined fibers into SP may reflect differences in the structure of SP in different regions of the amygdala.

493.15

[^{3}H]INOSITOL 1,4,5-TRISPHOSPHATE BINDING IN NORMAL AND ALZHEIMER DISEASED BRAIN. L.T. Young*, P.P. Li, S.J. Kish*, A.S. Chiu* and J.J. Warsh (SPON: Y. Israel). Clarke Institute of Psychiatry, Toronto, Ontario, CANADA M5T 1R8.

The phosphatidylinositide generated second messengers, especially inositol 1,4,5-trisphosphate (IP_3), are important in neuronal transmembrane signal transduction. IP_3 regulates brain calcium metabolism by binding to endoplasmic reticulum and plasma membrane to mobilize calcium stores. Binding sites for [^{3}H]IP_3 have recently been demonstrated in membranes prepared from rat brain which likely represent putative physiologically significant IP_3 receptors. In view of the importance of IP_3 in CNS calcium metabolism and neuronal function, it is of interest to examine [^{3}H]IP_3 binding sites in the normal and diseased human brain.

Membranes were prepared from autopsied cerebral cortices obtained from six subjects who were free from psychiatric or neurological disease. [^{3}H]IP_3 was found to bind with an affinity of 27 ± 8 nM (mean ± SEM); capacity (B_{max}) of 1.09 ± 0.18 pmol/mg protein and was reversible in the presence of an excess of unlabelled IP_3. This is the first demonstration of specific, saturable, and reversible binding of [^{3}H]IP_3 to membranes prepared from human brain. These [^{3}H]IP_3 binding sites are likely to be physiologically significant receptors. Data on brain regional distribution in subjects with Alzheimer's disease and age matched controls will also be presented.

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493.16

TETRAHYDROAMINOACRIDINE IS CONCENTRATED IN BRAIN FOLLOWING INTRAPERITONEAL ADMINISTRATION. D. Liston, L. Russo*, E.E. Mena and I. Williams*, Pfizer Central Research, Groton, CT 06340.

THA is a cholinesterase inhibitor that is currently undergoing clinical trials for use in Alzheimer's Disease. We have examined some properties of THA *in vitro* and *in vivo* to define the mechanism by which THA produces its therapeutic effects. *In vitro*, THA inhibits acetylcholinesterase (AChE) from rat brain and human erythrocytes with an IC50 of 220 nM. The kinetics of enzyme inhibition are best fitted by a model incorporating mixed competitive and non-competitive inhibition, with a K_{ii}=246 nM a K_{is}= 145 nM. THA is considerably more potent at inhibiting butyrylcholinesterase, with an IC50=9 nM. We examined the ability of THA to displace a number of ligands from rat brain membranes. Binding to cholinergic receptors was weak, with 50% displacement of ^{3}H-QNB (muscarinic) at 5.2 uM, ^{3}H-AFDX-116 (M2) at 1.7 uM, ^{3}H-telenzepine (M1) at 10 uM and ^{3}H-nicotine at >10 uM. THA displaces ^{3}H-prazosin (a_1 adrenergic) with an IC50 of 3.3 uM and ^{3}H-mepyramine (H1 histamine) with an IC50 of 5 uM. Monoamine oxidase from brain or liver was not significantly inhibited by THA below 100 uM. *In vivo*, THA exhibited an unusual distribution. Following 3.2 mg/kg i.p., a dose active in mouse passive avoidance, THA was 10-fold higher in brain than in plasma from 20-120 min, with the highest brain concentration (at 20 min) of 2.4 uM. At 120 min, brain THA was 0.34 uM, well above the IC50 for inhibition of AChE. A monohydroxylated metabolite of THA was observed in brain and plasma; this metabolite was a weak inhibitor of AChE. We conclude that the inhibition of brain AChE by THA is sufficient to explain its therapeutic action in Alzheimer's Disease.

493.17

THA INCREASES ACTION POTENTIAL DURATION OF CENTRAL HISTAMINE NEURONS IN VITRO. P.B. Reiner and E.G. McGeer. Kinsmen Laboratory, Dept. Psychiatry, Univ. of British Columbia, Vancouver, BC Canada V6T 1W5.

THA (9-amino-1,2,3,4-tetrahydroacridine) has been reported to produce marked clinical improvement in patients suffering from Alzheimer's disease (AD). THA has anticholinesterase activity and so might alleviate the well-documented deficit in cortical cholinergic innervation in AD. However, THA appears to be clinically more effective than other anti-cholinesterase agents, and thus other mechanisms might play a role in its therapeutic efficacy.

Intracellular recordings were obtained from histaminergic tuberomammillary neurons recorded in vitro. THA in concentrations as low as 1 μM increased the duration of the action potential, and this effect was not mimicked by physostigmine. The many similarities between hypothalamic histamine neurons and other central aminergic neurons suggest that THA may have similar effects upon these neurons as well. By its effect upon action potential duration, THA may increase release of amines upon the cortical targets of aminergic neurons which degenerate in AD. Thus, the therapeutic efficacy of THA may derive from a combination of its anticholinesterase activity and its effects upon the duration of action potentials of aminergic neurons.

493.18

ALTERATIONS IN RESPONSE TO ETHANOL BY TACRINE AND PHYSOSTIGMINE A. Rashtis*, S. Childres*, K. Tachiki*, C. Melchior, A. Steinberg*, & R. F. Ritzmann. Sepulveda & Brentwood VAMC, & Olive View/UCLA MC & CSU Northridge. LA, CA 91343.

Tacrine (THA), a less potent inhibitor of AChE than physostigmine (PHY), has been reported to be more effective in treating Alzheimer's disease. This suggests a pharmacokenetic difference or a different mechanism of action. Rats were injected with THA (3mg/kg) and 2 hrs later various brain regions were isolated and THA and amine levels were determined. In mice, THA and PHY (0.125mg/kg) were injected 1 hr prior to an injection of ethanol (3.2 g/kg, ETOH) and sleep time was measured. The results indicate that THA, depending on brain region, is present in concentrations 50 to 500 times lower than the reported IC50 level for inhibiting AChE. While PHY lowers the duration of ETOH-induced sleep time, THA significantly increased sleep time. This suggests that THA is acting by some other mechanism. Preliminary results indicate that THA produced alterations in brain amines and their metabolites in specific brain regions. 5-HT and dopamine increased, depending on the brain region, as much as 200%. Norepinepherine in cortex decreased by 17%. (Funded by Vet. Admin.)

493.19

INFLUENCE OF FRONTAL CORTEX LESION BY IBOTENIC ACID ON DISCRIMINATION AVOIDANCE LEARNING IN RATS. C. Hara, N. Ogawa and T. Naruo*. Dept. Pharmacol., Ehime Univ. Sch. of Med., Ehime-ken 791-02, Japan.

The memory dysfunction of alzheimer disease has been associated with a cortical cholinergic deficiency. In the rat, the ibotenic acid lesion of the nucleus basalis magnocelluralis (NBM) reduces choline-acetyltransferase activity in the dorsolateral frontal cortex (DFC), medial prefrontal cortex (MPC) and parieto-temporal cortex. The present study examined influence of ibotenic acid lesion of DFC, MPC or NBM on retention of discrimination avoidance learning (DAL). Male Wistar strain rats(8 weeks old) were housed in an air-conditioned room (23±1°C) with 12: 12 LD cycle (lights on at 0700) under free access to food and water. In the conditioning, two pure tones with 800 Hz for positive conditioned stimulus (CS) and 400 Hz for negative CS, and visa versa, were used in the two compartment shuttle box. Bilateral DFC and MPC, and unilateral NBM were lesioned by isotenic acid (5 μg/μl for 2 min) under pentobarbital anesthesia (45 mg/kg, i.p.). The retention of DAL was examined on 7, 10, 14 and 21 days after the surgery. The DFC lesion impaired the retention accompanied with increased negative response (NR) without affecting positive response(PR). The MPC lesion elicited the impairment of DAL with increased NR and decreased PR similar to NBM lesion. These results suggest that local dysfunction of cortex region related with NBM elicits impairment of learning behavior.

493.20

REGULATION OF SYNAPTIC EFFICACY IN THE HIPPOCAMPUS BY PHOSPHOETHANOLAMINE. G. Barrionuevo, J.E. Bradler*, D. McKeag* and J.W. Pettegrew*. Depts. of Behavioral Neuroscience & Psychiatry & WPIC, University of Pittsburgh, Pittsburgh, PA 15260.

The phosphomonoester phosphoethanolamine (PEA), a key intermediate in the biosynthesis of membrane phospholipids, is released spontaneously and in a stimulus (high K^+ or kainate) dependent manner from the in vivo hippocampus. In addition, ^{31}P NMR spectra demonstrated elevated levels of PEA in Alzheimer's diseased brains. To investigate the effects of PEA on synaptic efficacy, extracellular recordings were made from rat hippocampal slices (3 month old Fischer 344). The amplitude of population EPSPs evoked by stimulating the Schaffer collateral/commissural input to CA1 neurons was monitored prior to and during a 30 min bath application of varying concentrations of PEA. At both 1 mM and 5 mM, PEA induced a substantial depression of population EPSP amplitudes (45% +/- 21 % depression, n=8). This depression was only partially reversible following extended wash periods (87% +/- 21 % of baseline at 30 min, n=8). ^{31}P NMR spectra from freeze-clamped hippocampal slices revealed no effect of PEA on high-energy phosphate or membrane phospholipid metabolism. We are currently studying this regulation with intracellular recordings. Supported by R01-AG05657, AG-05133-01A1, and R01-MH4158 to J.W.P., and an RCDA (NS01196) to G.B.

493.21

REGIONAL GLUCOSE METABOLISM ALTERATIONS WITH INTRAVENTRICULAR QUINOLINIC ACID ADMINISTRATION

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Neurodegenerative disorders may involve neurotoxic EAA transmitters like quinolinic acid (QA). In an attempt to develop an animal model of Alzheimer's disease, we have evaluated two different administration techniques, chronic intraventricular injection (7 days) and intraventricular infusion (14 days) in the rat, to test brain metabolic changes and its similarity to Alzheimer metabolism. QA was introduced into the right lateral ventricle using either daily injection of 10 ug QA via chronic intracerebroventricular cannula for 7 days, or continuous infusion of QA using osmotic minipump (Alzet 2002) for 14 days with a total delivery of 400 ug QA/rat. Local cerebral glucose utilization (LCGU) was measured in 55 different brain areas using the quantitative [^{14}C]-2-deoxyglucose (2DG) autoradiographic method (Sokoloff et al., 1977), as umol glucose/100 gm brain tissue/min. Statistical analysis was done with multivariate analysis of variance. No remarkable alterations in LCGU were noted in the daily QA injection animals. Animals with the 14 day QA infusion demonstrated LCGU in a number of cortical brain areas and increases in LCGU in selected sensory processing areas.

(N=4)	Saline	QA
Frontal Cortex	83.6 ± 5.1	65.6 ± 1.9*
Cingulate Cortex	96.9 ± 6.1	78.2 ± 2.7*

We will compare alterations in glucose metabolism in this rat model with metabolic alterations seen in Alzheimer patients.

493.22

[^{3}]MK-801 BINDING SITES IN ALZHEIMER'S DISEASE.

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Excitatory amino acids and their receptors, particularly the NMDA/PCP complex, have been implicated in the pathogenesis of Alzheimer's disease. In an attempt to further study this receptor complex, postmortem brain tissue homogenates from 22 Alzheimer patients were compared to 21 age-matched controls for binding sites of [^{3}]MK-801, a noncompetative antagonist of PCP binding to the NMDA receptor associated ionophore. Seven brain regions were studied: Brodmann areas A-4, A-9, A-39, A-22, A-38, A-17, and hippocampus. Binding studies were performed with Tris buffer using filtration method. There were no consistent differences between patients and controls in either binding density or affinity constants in any of the areas studied. Hippocampal binding sites correlated positively with symptom duration (r=.963) and age at death (r=.619) in Alzheimer subjects. Loss of perforant pathway afferents to the hippocampus in Alzheimer's disease might result in denervation supersensitivity. Although lack of changes in binding densities of the NMDA/PCP complex in Alzheimer's disease in this study may be due to methodological discrepencies, since the use of homogenized tissue might mask changes limited to microscopic elements seen with autoradiography, nevertheless, these findings lend no support to the view that cortical NMDA receptor-bearing neurons are preferentially affected in this disorder. However, the possibility that the presence of these receptors is a necessary, but hardly sufficient, basis for cell death cannot be excluded.

LEARNING AND MEMORY: ANATOMY III

494.1

LEARNING AND MEMORY DEFICITS AFTER LESIONS OF NUCLEUS BASALIS IN TURTLES.

A. S. Powers, A. Blau*, & M. Petrillo*. Dept. of Psychology, St. John's University, Jamaica, NY 11439.

The basal forebrain of turtles contains a nucleus basalis, a group of cholinergic cells that project to the dorsal cortex. The dorsal cortex is a three-layered cortex on the surface of the telencephalon. We compared the effects of lesions of nucleus basalis and dorsal cortex on pattern discrimination acquisition and reversal and on retention of maze learning. Lesions of the dorsal cortex were made by suction; those in nucleus basalis were made by injections of ibotenic acid. Compared to sham-lesioned controls, both lesions produced an impairment on acquisition and reversal of a horizontal-vertical discrimination. There was no significant difference between the two lesioned groups on either acquisition or reversal. Maze retention was also impaired by both lesions, but there was a suggestion that the impairment was greater in the nucleus basalis group. These results suggest that the nucleus basalis of mammals is a phylogenetically ancient structure that participated in learning and memory in the reptilian ancestors of mammals.

494.2

MAGNETIC RESONANCE IMAGING OF THE RHESUS MONKEY BRAIN: I. STEREOTAXIC LOCALIZATION OF THE BASAL FOREBRAIN.

T.G. Aigner, R.C. Saunders, and J.A. Frank. Laboratory of Neuropsychology, NIMH and Department of Radiology, NIH, Bethesda, MD 20892.

Subcortical neurosurgery in nonhuman primates has traditionally used stereotaxic atlases, x-ray radiography, or electrophysiological recording to locate targeted structures. In an attempt to improve on these sometimes unreliable methods, we used magnetic resonance imaging (MRI) in combination with a specially designed nonmagnetic stereotaxic instrument (SI) to locate the nucleus basalis of Meynert and the medial septum/diagonal band nuclei. These basal forebrain nuclei were chosen to validate the MRI/SI method because their irregular shape makes them especially difficult targets as judged by results with other methods. Monkeys were anesthetized, positioned in the SI, and then scanned in a General Electric Signa 1.5 Tesla MRI unit. Stereotaxic coordinates were derived using standard software routines on the MRI unit. For surgery, each animal was re-positioned in the SI and ibotenic acid was then injected into 14 basal forebrain sites. The animal was re-scanned within 2 weeks to visualize the lesion. Histological analysis confirmed the precision of the technique; 35 out of 42 injections in 3 hemispheres were direct hits and 7 were within 0.5 mm of their intended targets. MRI/SI appears to provide a level of accuracy and reliability surpassing that obtained with earlier methods.

494.3

MAGNETIC RESONANCE IMAGING OF THE RHESUS MONKEY BRAIN: II. RECONSTRUCTION OF EXPERIMENTAL LESIONS.

R.C. Saunders, T.G. Aigner and J.A. Frank*. (SPON: M. Haenlein) Dept. Radiology, NIH and Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

The importance of a neural region in a particular psychological function (e.g. memory) can be inferred after psychometric analysis is directly related to the underlying neuropathology. In monkeys, because of their high financial and training costs, this is often delayed until extensive investment in experimental testing is completed. We used magnetic resonance imaging (MRI) to determine the limits of experimental lesions in the rhesus monkey brain. MRI is uniquely suited for visualizing the brain in vivo due to its soft tissue contrast and the abilty to obtain images in three dimensions. Monkeys were appropriately anesthetized, positioned in a specially developed nonmagnetic stereotaxic instrument, and centered in a GE 1.5 Tesla MRI unit. Monkeys were scanned both pre- and post-operatively. The lesions were made by aspiration or by the excitatory neurotoxin ibotenic acid at subcortical and cortical sites. Histological analysis confirmed that both aspiration and neurotoxin lesions can be precisely delineated from MR images. This technique has the added advantages over classic histological assessment in that it allows the lesion to be visualized in any plane and it permits a non-invasive means for evidential histological analysis.

494.4

DIFFERENTIAL ANATOMICAL PROJECTIONS BETWEEN FRONTAL CORTICES AND THE BASAL FOREBRAIN IN THE RAT.

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Neural connections of the rat's basal forebrain and frontal cortices were studied using the retrogradely transported fluorescent tracer bis-Benzimide combined with acetylcholinesterase (AChE) histochemistry. Bis-Benzimide injections in the nucleus basalis magnocellularis labeled neurons in the dorsolateral frontal cortex. In contrast, injections in the horizontal nucleus of the diagonal band labeled neurons in the medial prefrontal cortex. Conversely, when bis-Benzimide was injected in the medial prefrontal or dorsolateral frontal cortices, labeled neurons were seen in the horizontal nucleus of the diagonal band or the nucleus basalis magnocellularis, respectively. Identification of these basal forebrain areas was confirmed by the AChE stain. These results indicate that the basal forebrain of the rat receives reciprocal differential anatomical projections from frontal cortices and this suggests that these connections may mediate differential behavioral functions.

494.5

NUCLEUS BASALIS MAGNOCELLULARIS IS INVOLVED IN TASTE AVERSION LEARNING IN RATS. R. Tardif*, R.P. Kesner, & R.F. Berman. (SPON: S.W. Miller). Departments of Psychology, University of Utah, Salt Lake City, Utah 84112 and Wayne State University, Detroit, Michigan 48202.

It has been shown that the basolateral amygdala plays an important role in mediating taste aversion learning. Other studies suggest that the nucleus basalis magnocellularis (NBM) provides critical cholinergic input pathways to the basolateral amygdala.

Long-Evans rats received either ibotenic acid lesions of the NBM, vehicle control injections, electrolytic lesions of the basolateral amygdala, or served as nonoperated controls. Nonoperated controls, vehicle injected controls, and unilateral NBM lesioned animals all displayed taste aversion learning. Animals with bilateral lesions of the NBM or basolateral amygdala exhibited a disruption in taste aversion learning. Additional analysis of ChAT content in amygdala revealed that there was a significant negative correlation between the degree of disruption of taste aversion learning and levels of ChAT in amygdala. These data suggest that even though NBM lesions produce a taste aversion learning deficit that parallels impairments seen in animals with basolateral amygdala lesions, this deficit does not appear to be mediated by cholinergic projections to the amygdala. It is suggested that NBM lesions might have damaged other cholinergic systems or that some other neural transmitter projecting to amygdala might be involved.

494.6

BEHAVIORAL EFFECTS OF ELECTROLYTIC VERSUS IBOTENATE LESIONS OF NUCLEUS BASALIS IN RESPONSE TO GANGLIOSIDE TREATMENT. B.S. Bitran*, L. Lescaudron*, and D.G. Stein. (SPON: M. Wilson). Brain Research Lab., Clark University, Worcester, MA 01610.

Lesions of the nucleus basalis magnocellularis (NBM) deplete cortical acetylcholine levels and produce memory impairments in rats, while GM_1 ganglioside treatment stimulates the recovery of those levels after lesions of the NBM (Florian et al. *Neurosci. Lett.* 75: 313-316, 1987). In the present study, electrolytic but not ibotenate lesions of the NBM produced deficits in the Morris Water Maze; GM_1-treated rats were not significantly different from saline-treated rats on this task. Both electrolytic and ibotenate lesions impaired acquisition of a passive avoidance task; however, GM_1 treatment facilitated performance of ibotenate-lesioned rats only. Locomotor activity, measured in an open field, was not affected by NBM lesions or by GM_1 administration. Although the two lesion types did not differ in terms of the number of NBM cells destroyed, the electrolytic lesions produced greater damage to adjacent structures. GM_1 treatment did not affect cell survival in the NBM after electrolytic or ibotenate lesions. Further correlations between behavioral and neuroanatomical data will be reported.

Supported by grants from Fidia Research Laboratories and S. & C. Del Duca Foundation.

494.7

DIFFERENTIAL EFFECTS OF QUISQUALATE OR IBOTENATE INJECTED INTO NUCLEUS BASALIS MAGNOCELLULARIS (NBM) ON BEHAVIOR AND NEUROCHEMISTRY. G.L. Wenk, A. Markowska* and D.S. Olton. Dept. of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Lesions of the NBM induced by either quisqualic (QUIS) or ibotenic (IBO) acid produced different effects on behavior and neurochemistry in rats. IBO injections decreased choline acetyltransferase (ChAT) activity (48%) and [^{3}H]Neurotensin binding (45%) in frontal cortex and impaired performance in T-maze alternation. QUIS injections decreased ChAT activity (78%) but did not alter [^{3}H]Neurotensin binding or impair performance. Therefore, non-cholinergic cells that are in the NBM, project to cortex, and have presynaptic [^{3}H]Neurotensin binding sites may be involved in the performance of this task. These data have implications for the effects of loss of NBM cells and provide new information about the neural organization of cells in the NBM and the mechanisms responsible for their degeneration in Alzheimer's Disease.

494.8

NUCLEUS BASALIS LESIONS AND PAVLOVIAN CONDITIONING IN THE RABBIT. S.R. Ginn and D.A. Powell. Dorn VA Hospital and University of South Carolina, Columbia, SC, 29201.

Ibotenic acid (IA) lesions of the nBM were made prior to classical conditioning in rabbits; other rabbits were subjected to sham lesions of the nBM, served as unoperated controls, or received pseudoconditioning (viz., random shocks/tones). Eyeblink (EB) and heart rate (HR) conditioned responses (CRs) were assessed. Lesions of the nBM had no effect on acquisition of the EB CR, but the HR CR of the lesioned animals was attenuated compared to the control groups. IA lesions of the nBM reduced cortical CAT concentrations by 50% compared to control animals. In a second experiment, rabbits received sham or nBM lesions after conditioning training was completed, but prior to retention testing. There were no differences between the groups for the HR or EB CRs during either phase of this experiment, even though IA lesions reduced cortical CAT concentrations by 80%.

These results suggest that the nBM may be part of a cortical-subcortical pathway that modulates Pavlovian autonomic conditioning. The effects of the lesions of the nBM were most apparent early in conditioning, suggesting that the nBM affects attention/stimulus registration processes during learning.

494.9

THE ROLE OF THE CENTRAL NUCLEUS OF THE AMYGDALA AND ITS EFFERENT PATHWAY TO THE BRAINSTEM IN SHOCK SENSITIZATION OF THE STARTLE REFLEX. J.M. Hitchcock, C.B. Sananes & M. Davis. Dept. of Psychiatry, Yale Univ., Ribicoff Res. Fac., Conn. Mental Health Ctr., New Haven, CT, 06508.

The amplitude of the acoustic startle reflex can be markedly increased during the 5-40 minutes following presentation of ten 0.6 mA footshocks (shock sensitization). This facilitation represents an unconditioned effect of shock on startle. A consistent literature indicates that the amygdala plays an important role in unconditioned as well as conditioned responding to fear-producing stimuli.

Lesions of the central nucleus of the amygdala, but not of the immediately adjacent lateral nucleus, blocked shock sensitization. Central nucleus lesions also decreased reactivity to shock (jumping and flinching). However, this did not account for the blockade of shock sensitization, because when a higher shock intensity was used, producing equivalent reactivity to that of controls at the lower intensity, central nucleus lesions still blocked shock sensitization.

A follow-up study was conducted to determine the central nucleus efferent pathway that might be involved in the mediation of shock sensitization of startle. Lesions of the rostral part of the ventral amygdalofugal pathway (VAF), which carries efferents to the forebrain, had no effect on shock sensitization of startle. In contrast, lesions of the caudal part of the VAF, a branch of which projects directly to the startle circuit, blocked shock sensitization of startle.

These findings, along with previous results, lead us to hypothesize that activation of the central nucleus of the amygdala increases startle through its projection to the startle pathway, and this mediates the unconditioned effects of shock on startle, as well as the conditioned effects of stimuli paired with shock on startle.

494.10

LESIONS OF THE BASOLATERAL AMYGDALA AND OF THE PYRIFORM CORTEX IMPAIR ACQUISITION OF A CONDITIONED ODOR PREFERENCE. N. Beaulieu*, R. Morris* and M. Petrides. (SPON: D. N. Pandya). Dept. of Psychology, McGill University, Montreal, Quebec, Canada, H3A 1B1.

Previous research demonstrated that lesions of the basolateral amygdala (BLA) and of the pyriform cortex (PC) impaired the acquisition of a conditioned odor aversion (Beaulieu et al., Soc. Neurosci. Abstr., 1987). The present study investigated the effects of similar lesions on the acquisition of a conditioned odor preference.

Testing was carried out in a chamber with a drinking spout at each end of it. Water-deprived rats were exposed simultaneously to 2 distinct odors (chocolate and almond) for 15 min./day for 10 consecutive days. One of the odors (S+) was present in the shavings surrounding one of the spouts from which a 15% sucrose solution was delivered, while the other odor (S-) surrounded an empty spout. Pairing conditions and side of presentation of the odors were counterbalanced. On day 11, the rats were again exposed to the odors but now water was available from both spouts. Normal animals drank more water from the spout on the side with the S+ odor, demonstrating a preference for that odor. Both groups of operated animals drank similar amounts of water from both spouts, indicating a lack of odor preference.

These results show that, with the present testing procedures, lesions of both the BLA and the PC impair formation of a preference for an odor. However, the contribution of these 2 structures may not be of the same nature.

494.11

The amygdala is involved in the expression of conditional analgesia. F.J. Helmstetter, R.N. Leaton, M.S. Fanselow*, & D.J. Calcagnetti* Psychology Department, Dartmouth College, Hanover, N.H. 03755

The amygdala seems to play an important role in the acquisition and performance of conditional fear as reflected in a number of preparations. We wished to determine if this structure is involved in the analgesia displayed by rats in the presence of shock associated stimuli. Relative to controls, animals with electrolytic lesions involving portions of the central, lateral, and basolateral amygdaloid nuclei spent less time freezing and were less analgesic as indexed by the formalin test. Bilateral microinjection of diazepam (30µg /1µl x2) into the amygdala produced a similar pattern of results. These data are consistent with the position that conditional analgesia is a response to a central fear-like motivational process.

494.12

SUBCORTICAL AFFERENT CONNECTIONS OF THE AMYGDALOID CENTRAL NUCLEUS IN RABBITS. C.G. Gentile*, C.G. Markgraf, P.M. McCabe, D.R. Liskowsky, R.W. Winters*, & N. Schneiderman (SPON: M.D. Gellman). Dept. of Psychology, Univ. of Miami, Coral Gables, FL 33124.

The amygdaloid central nucleus (ACE) is known to play an important role in classically conditioned heart rate responses to acoustic stimuli in the rabbit (Kapp *et al.*, 1979; Gentile *et al.*, 1986). While the efferent projections of this nucleus to various cardioregulatory structures have been examined extensively in this and other species, afferent projections to the ACE have been described in one study in the rabbit (Kapp et al., 1984). The present study examined subcortical afferent projections to the ACE and other amygdaloid nuclei, with a focus upon inputs from auditory nuclei.

New Zealand rabbits received injections of the retrograde fluorescent tracer Fluoro-gold (4% in saline, 20nl) in ACE. Two to three weeks later, the animals were perfused and sections were examined, using fluorescent microscopy, for the presence of retrogradely labelled cells in subcortical structures. Labelled neurons were located primarily ipsilaterally, in several areas including substantia innominata, midline thalamic nuclei, ventromedial and paraventricular hypothalamus, dorsal and ventral periacqueductal gray region, medial parabrachial nucleus, substantia nigra, locus coeruleus, and nucleus tractus solitarius. Of particular interest were labelled neurons in the magnocellular region of the medial geniculate nucleus and in the ventrolateral parabrachial region bordering on the intermediate nucleus of the lateral lemniscus. Both groups of cells may relay auditory or multi-modal sensory information to the ACE. Supported by NIH grants NS 24874, HL 07426, and HL 36588.

494.13

PROJECTIONS FROM THE ACOUSTIC THALAMUS TERMINATE IN THE LATERAL BUT NOT CENTRAL AMYGDALA. *C.F. Farb, D.A. Ruggiero and J.E. LeDoux* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Acoustic stimuli are transformed into symbols of danger by way of projections from the acoustic thalamus to the amygdala. The relay to the amygdala should, therefore, originate in thalamic areas that are within the projection field of the inferior colliculus (IC). In the present study we determined whether the central (CA) and/or lateral (LA) amygdaloid nuclei receive inputs from neurons that receive afferents from the IC. WGA-HRP was delivered iontophoretically to CA (n=8) or LA (n=10). Injections confined to LA resulted in transport to cells in the posterior intralaminar nucleus (PIN) and medial division of the medial geniculate body (MGM). Following injections in CA labelled neurons were located in the posterior thalamic region (PT) between the MGM/PIN and the anterior pretectal nucleus. Injection of WGA-HRP into the MGM/PIN (n=2) resulted in retrograde transport to cells in the shell regions of IC and anterograde transport to LA. Injections in PT (n=4) failed to label cells in IC, but did produce anterograde transport to the CA. Injections of IC (n=5) produced anterograde transport to the MGM/PIN, but not to PT. These observations demonstrate that neurons in the MGM/PIN receive inputs from the IC and project to LA. In contrast, CA does not appear to receive a direct input from the acoustic thalamus. The projection to LA may, therefore, be a critical relay in emotional learning. (Supported by MH38774).

494.14

SHORT LATENCY ORTHODROMIC ACTION POTENTIALS EVOKED IN AMYGDALA AND CAUDATE-PUTAMEN BY STIMULATION OF THE MEDIAL GENICULATE BODY. *C. Clugnet, J.E. LeDoux, S.F. Morrison and D.J. Reis* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Anatomical tracing studies indicate that the medial geniculate body (MGB) projects to areas of the amygdala and caudate-putamen, as well as to the auditory cortex. Responses of single neurons to electrical stimulation of the MGB were examined in those subcortical areas that receive direct anatomical projections from the MGB. Recordings were made in rats (n=20) anesthetized with chloral hydrate (7%, ip). Units in the caudate-putamen and lateral amygdala were excited by short pulses (twin shocks, 60 µsec each, 200 µsec apart, 500 µA, 0.1 Hz). Mean latencies were 3.7±1.7 msec in the lateral caudate-putamen (n=61), 5.3±2.2 msec. in the medial caudate- putamen (n=46), and 7.2±2.6 msec. in the lateral amygdala (n=35). Units in other amygdaloid regions required longer, higher frequency pulses (single shock, 500µsec, 500 µA in the central amygdala and 1.3 mA in the basolateral/basomedial amygdala, with frequencies varying from 0.2 to 1 Hz). Mean latencies were 9.9±2.6 msec in the central amygdala (n=38) and 13.3±3.7 msec in the basal amygdala (n=50). In the latter regions, responses followed a caudo-rostral gradient, with cells responding to MGB stimulation being more numerous caudally. These data are consistent with anatomical findings demonstrating direct projections to the lateral amygdala and caudate-putamen. The longer latency and higher threshold responses in the central and basal amygdaloid nuclei may be accounted for by multisynaptic projections to these areas, perhaps from one of the regions receiving direct MGB projections. Supported by NYHA and MH38774.

494.15

CONTEXTUAL MODULATION OF EMOTIONAL PLASTICITY. *A.E. Xagoraris, L.M. Romanski, B.T. Volpe and J.E. LeDoux*. Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

When rats experience an acoustic or visual stimulus in the presence of footshock, the acoustic or visual stimulus becomes a conditioned emotional stimulus (CS) capable of eliciting learned fear responses. We have found that the pairing of one CS (CS1) with a footshock unconditioned stimulus (US) prevents the subsequent development of conditioned responses to a second CS (CS2) paired with the same US one week later in the same apparatus. Conditioning with CS2 is also interfered with if CS1 is presented in a random relation to the US or if only the US is given during the first training session. However, conditioning to CS2 is unaffected when CS1 and CS2 are paired with the US in physically unique conditioning chambers. These findings suggest that once the rat is shocked in a given apparatus, the apparatus becomes a contextual CS and that novel stimuli subsequently paired with the US in that particular environment are ignored. This phenomenon is similar to "blocking", where prior training with CS1 interferes with subsequent training with a compound CS consisting of CS1 and CS2. However, in the present case the blocking effect is not due to the prior pairing of CS1 with the US but to a relation that develops between the US and environmental context in which it first appears. Context also affects the expression of fear, since the magnitude of the fear response elicited by CS1 is smaller when tested in a novel chamber than in the conditioning apparatus. (Supported by MH38774).

494.16

DESTRUCTION OF PERIRHINAL AND NEOCORTICAL PROJECTION TARGETS OF THE ACOUSTIC THALAMUS DOES NOT DISRUPT FEAR CONDITIONING. *L.M. Romanski, A.E. Xagoraris, D.J. Reis and J.E. LeDoux*. Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Destruction of the medial geniculate body (MGB), but not its neocortical projection field, disrupts the classical conditioning of autonomic activity (increases in arterial pressure, AP) and emotional behavior ("freezing", F) to a pure tone paired with footshock. However, cortical areas along the rhinal fissure, which were spared in previous lesion studies, also receive inputs from MGB. In the present study we therefore examined whether combined removal of perirhinal and neocortical projection areas would affect emotional (fear) conditioning. Rats were lesioned, allowed to recover for 2-3 weeks, subjected to fear conditioning, and tested. Conditioned responses did not differ in controls (AP, 16±2; F, 117±2; n=7) and in rats with cortical ablations (AP, 13±1; F, 98±16; n=6). In contrast, conditioned responses were significantly reduced by lesions of MGB (AP, 4±1, $p<.01$; F, 0; $p<.01$; n=7). Conditioned responses were similar in animals with MGB lesions to responses in controls given random presentations of tone and shock (AP, 4±1.5, ns; F, 4±3, ns; n=7). These data demonstrate that fear conditioning does not depend upon any cortical area receiving inputs from the MGB. Since the MGB is necessary for the formation of the association between the tone and shock, its subcortical projections, which originate in the medial MGB and posterior intralaminar nucleus, must be essential components of the fear conditioning circuitry. (Supported by MH38774).

494.17

ANIMALS WITH ISCHEMIC OR IBOTENIC ACID HIPPOCAMPAL INJURY DISPLAY SPATIAL MEMORY IMPAIRMENT. Bruce T. Volpe, Beverly Waczek*, P. Colombo, H.P.Davis. Dept. of Neurology, Cornell Univ. Med. Ctr., White Plains, NY 10605, Univ. of Col., Col. Springs, Col.

Animals exposed to ischemia by the four vessel occlusion method (post ischemic, PI animals) reproducibly develop severe injury to the CA1 hippocampus and dl caudate, and have a spatial working memory impairment on radial 8, 12 and modified T-mazes. We tested whether ischemic hippocampal injury was sufficient to cause a spatial working memory impairment by comparing the performance of PI animals and animals exposed to high (hi-IBO,16-24 mcg) and low dose (lo-IBO,6-12 mcg) ibotenic acid injections into the dorsal hippocampus. We used a split stem T-maze to measure memory for invariant, trial independent information (reference), and memory for variable trial dependent information (working). Reference performance required the animals to choose either the rough or smooth surfaced alleyway on the stem. Correct working performance required that the animal alternate choice of goal arm for a food reward. Animals were trained for 20 trials, exposed to ischemia or ibotenic acid and returned for 30 post operative trials. Results showed no difference in pre-operative performance ($p>.5$) among all groups. On post operative trials there was no difference among groups on reference memory performance ($p>.1$). Lo-IBO animals performed the working memory task comparably with IBO controls ($p>.1$). However, both PI and hi-IBO animals performed the working memory task worse than controls ($p<.01$). This data suggests that hippocampal ischemic injury may be sufficient to cause the spatial working memory impairment in PI rats, and, further, suggests that this deficit may be a function of the amount of hippocampal injury.

494.18

EFFECTS OF DORSAL CAUDATE DAMAGE ON MEMORY FOR INVARIANT SPATIAL AND TACTILE INFORMATION. P.J. Colombo, H.P. Davis, B.T. Volpe. Dept. Psychology, Univ. Colorado at Colorado Springs, 80933, Dept. Neurology Cornell Univ. Med. Ctr., White Plains, NY 10605.

Electrical stimulation or lesions to the caudate impairs acquisition and retention in a variety of tasks that have an invariant rule based component (Phillips & Carr, Cand. J. Neurol. Sci., 14, 1987). Rats were pretrained on a 12-arm radial maze with 7 of 12 arms baited for either 36 or 80 daily trials, subjected to bilateral radiofrequency lesion of the dorsal caudate, allowed to recover, and then tested. Working performance, remembering which arms have been entered on a particular trial, was not impaired in rats given either 36 or 80 pretraining trials. However, the invariant reference aspect of radial maze performance was impaired in rats pretrained for 36 trials ($p<.01$). Reference performance was not impaired in animals given 80 pretraining trials ($p>.20$). Rats with caudate damage were also tested in a split stem T-maze that required the animal to make a tactile and spatial discrimination. The tactile discrimination did not vary across trials whereas the spatial discrimination varied from trial to trial. Caudate and control rats demonstrated similar performance on both the tactile and spatial discrimination. The results indicate that the dorsal caudate is initially required for normal reference performance in a spatial task. After extensive pretraining the caudate is not necessary for accurate reference performance in the radial maze.

494.19

PREOPERATIVE TRAINING EFFECTS ON RADIAL MAZE PERFORMANCE IN ANIMALS WITH ISCHEMIC OR IBOTENIC ACID HIPPOCAMPAL INJURY. H.P. Davis, P.J. Colombo, B.T. Volpe. Dept. Psychology, Univ. Colorado at Colorado Springs, 80933, Dept. Neurology, Cornell Univ. Med. Ctr., White Plains, NY 10605.

Rats with severe damage to the CA1 hippocampus following 30 minutes of ischemia by the 4 vessel occlusion method are impaired on the working memory aspect of a radial maze, but not on the reference aspect. Pretraining effects on working and reference performance were examined by training animals for either 36 or 80 trials prior to ischemic insult on a radial maze with 7 of 12 arms baited. Postoperative reference performance, as indicated by not entering unbaited arms, was not impaired in post ischemic rats given 36 or 80 pretraining trials. Working performance, reentering arms, was impaired in post ischemic rats given 36 pretraining trials ($p<.01$), but not in animals given 80 pretraining trials. Pretraining effects and task difficulty were further investigated in animals with ibotenic acid induced hippocampal damage by assessing working performance after 0, 30, or 60 pretraining trials on either a 4-, 8-, or 12-arm radial maze with all arms baited. Rats with hippocampal damage given 0 pretraining trials and tested on either a 4-, 8-, or 12-arm maze were impaired ($p<.05$). Rats given 30 pretraining trials demonstrated impaired working performance only on the 12-arm maze ($p<.05$). Rats pretrained for 60 trials demonstrated normal postoperative working performance. These findings show that pretraining and task difficulty significantly effect memory performance of animals with ischemic or ibotenic acid induced hippocampal damage.

494.20

OPPOSITE EFFECTS OF LEARNING AND RELEARNING OF A MAZE ON [^{3}H]2-DEOXYGLUCOSE UPTAKE: LIMITATIONS OF THE RELATIVE OPTICAL DENSITY MEASURE. M. Sarter (SPON: W.Kehr). Dept. Neuropsychopharmacology, Schering AG, D-1000 Berlin 65, F.R.G.

Rats were trained either in a 6-arm radial maze, or they were allowed to explore an alley maze with similar extensions or only the center of the maze. Following 8 acquisition sessions, a 5 day-break was followed by implantation of catheters into the jugular vein. Two days later, 2-deoxyglucose was injected 5 minutes before the test session. Controls were again tested in the maze center, the other two groups explored the 6-arm radial configuration. Rats which previously learned this configuration (relearners) showed clear evidence of memory compared to rats which experienced this configuration for the first time (learners). Glucose uptake was measured using the relative optical density measure. Compared to controls, relearners showed a general decrease of glucose uptake, whereas learners showed an increase of glucose uptake in limbic cortical and subcortical areas. Since relative optical densities vary considerably with subtle changes in white matter optical density, results based on the relative optical density measure may not be valid.

494.21

AFFERENT CONNECTIONS OF MAGNOCELLULAR REGION OF THE MEDIAL GENICULATE NUCLEUS IN THE RABBIT. P.M. McCabe, C.G. Markgraf, J.A. Quetel*, D.R. Liskowsky, R.W. Winters*, and N. Schneiderman. Dept. of Psychology, Univ. of Miami, Coral Gables, FL 33124.

The magnocellular region of medial geniculate nucleus (mMGN) has been demonstrated to be an important region for the acquisition and retention of classically conditioned cardiovascular and behavioral responses to aversive stimuli. Neurons in mMGN exhibit broad tuning curves, receive multi-modal sensory input, and show learning related changes in electrophysiological activity. Although the afferent connections of this region have been examined in other species (e.g. Ledoux *et al.*, 1985), inputs to mMGN have not been described in the rabbit. The present study sought to advance our understanding of the neural substrates of conditioning by assessing the afferent anatomical connections of mMGN in rabbits.

Pressure injections of the retrograde fluorescent tracer Fluoro-gold were made via a micropipette into mMGN or nearby control sites. Two weeks later the animals were perfused and sections were examined using fluorescence microscopy. Cell bodies were localized in several ipsilateral auditory structures including deep layers of the auditory cortex, the external nucleus of inferior colliculus (IC), the ventral portion of central nucleus of IC, the dorsal nucleus of the lateral lemniscus (NLL), and the ventral NLL. Dense labeling was also observed in the reticular nucleus of the thalamus, and more sparce labeling was seen in the deep layers of superior colliculus and in the spinal trigeminal nucleus. The mMGN connections we observed in rabbits are consistent with those found in rats. Supported by NIH grants NS 24874, HL 07426, and HL 36588.

494.22

THALAMIC INPUT TO MEDIAL PREFRONTAL CORTEX IN MACAQUES. X.-C.M. Lu*, J. Bachevalier, D. Kowalska*, and L.G. Ungerleider (SPON: B.M. Slotnick). Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

To determine the source of thalamic inputs to the medial aspect of the prefrontal cortex, we injected retrograde tracers (WGA-HRP, nuclear yellow, and/or bisbenzimide) into 8 medial prefrontal sites and tritiated amino acids into 6 thalamic sites, in a total of 9 rhesus monkeys. Two of the thalamic injections were confined to the magnocellular portion of the medial dorsal nucleus (MDmc), two were centered in MDmc but also included the midline nuclei, one was confined to the anterior nuclei (AN), and one was centered in AN but also included dorsal MDmc and dorsal MDpc (the parvocellular portion of MD). The results indicated that precallosal cortical areas 14, 25, and 32 all receive projections largely from dorsal MDmc. In the most anterior portions of areas 14 and 32 and in area 10, the projections arise from dorsal MDpc. Sub- and supracallosal area 24 receives its major thalamic input from AN (mostly the anterior medial nucleus). Projections from AN extend to large portions of medial limbic cortex, including, in addition to area 24, areas 23, 25, and retrosplenial and entorhinal cortex. Combining these results with prior data, we conclude that precallosal regions have access to amygdalar information via MDmc, while sub- and supracallosal regions have access to hippocampal information via AN.

494.23

VISUAL DISCRIMINATION LEARNING BY MONKEYS WITH MEDIAL THALAMIC LESIONS. S. Jacobson, N. Butters and E.C. Gower. VA Medical Center, Boston, MA 02130.

In a previous experiment, monkeys (M. fascicularis) with medial thalamic lesions were found to exhibit an acquisition deficit for a visual pattern discrimination task (A+ B-). The possibility that this deficit could be due to difficulty in reversing the rule which had been practiced in an immediately preceeding series of non-matching tasks (win/shift, with respect to the sample stimulus) was assessed by presenting 3 additional sets of discrimination problems. Each set was composed of 8 problems learned in succession to a criterion of 90% correct in 30 trials. Practice with associative problems did not reduce the performance decrement of the experimental monkeys, and it was apparent that they were retarded in developing a learning set. A transfer task (A+ C-, D+ B-) was also presented, in which the elements A+ and B- had been learned as a pair and maintained the original reward assignments as members of new pairs. Monkeys with lesions were not able to maintain the learned response choice as well as normal monkeys, when the context provided novel stimuli which were differentially rewarded. These results suggest that the deficit sustained by monkeys with medial thalamic lesions is related to the acquisition and maintainance of the stimulus/reinforcement association, and is affected by the amount of within-problem interference. (Supported by VA Medical Research funds.)

494.24

THE EFFECTS OF VENTRAL THALAMIC LESIONS ON COGNITIVE AND MOTOR COMPONENTS OF LEARNING IN THE RAT. R. Shen* and D. Asdourian. Dept. of Psych., Wayne state Univ., Detroit, MI 48202.

Recent evidence suggests that the ventromedial and ventrolateral nuclei of the thalamus(VM-VL) are important both in helping to maintain motor responses initiated from the motor cortex and in gathering critical subcortical information from the cerebellum and basal ganglia(BG) which it then sends to the cerebral cortex. The VM-VL is important because it is the only place through which subcortical information from BG and the cerebellum can be sent back to the cerebral cortex. Evidence also shows that the caudate-putamen(Cd-Pt) of the BG as well as the cerebellum play important roles in learning. Cd-Pt is found to be involved in visual and spatial discrimination learning and different paradigms of maze learning. The purpose of this study is to investigate the general neurological deficits and learning deficits produced by the electrolytic lesions of the VM-VL. A 15 item neurological test and 3 learning tasks: T-maze spatial reversal, T-maze brightness discrimination, and complex maze, were used in this study. No deficits were observed on any of the neurological test items following the VM-VL lesions. Lesions affected performance on the T-maze spatial reversal task (lamda ratio=3.21, df=4,20, p=.0345) but not on the other two learning tasks. The results were similar to those obtained by others in studies of the Cd-Pt.

494.25

PARASAGGITAL THALAMIC KNIFE CUTS RETARD PAVLOVIAN EYEBLINK CONDITIONING AND ABOLISH THE TACHYCARDIAC COMPONENT OF THE HEART RATE CONDITIONED RESPONSE. S.L. Buchanan and D.A. Powell. Neuroscience Lab, VA Medical Center and University of South Carolina, Columbia, SC.

Rabbits received parasaggital knife cuts lateral to the mediodorsal nucleus of the thalamus (MD), severing afferents and efferents to and from the prefrontal cortex. These animals were compared to sham animals in a Pavlovian eyeblink and heart rate conditioning experiment in which a tone was the conditioned stimulus and paraorbital shock was the unconditioned stimulus. Knife cuts retarded acquisition of the eyeblink conditioned response (CR), and abolished the late-occurring tachycardiac component of the heart rate CR. These data are compatible with previous experiments, which suggest that MD participates in the sympathetic control associated with somatomotor learning.

(Supported by VA Institutional Research Funds)

494.26

SPATIAL MEMORY DEFICITS FOLLOWING MAMMILLARY BODY LESIONS IN MICE ARE DEPENDENT OF THE EFFORTFUL REQUIREMENTS OF THE TASK. D.J. Beracochea and R. Jaffard, Lab. Psychophysiologie, UA CNRS 339, Université de Bordeaux I, Avenue des Facultés 33405 TALENCE FRANCE.

We have previously shown that electrolytic lesions of the mammillary bodies (MM) in mice induce memory deficits in tasks based on spontaneous alternation (S.A.) in a T-maze. Subsequent experiments showed that these deficits could be alleviated by using between-trials contextual changes. This suggests that the observed impairments might stem from the automatic (as opposed to effortful) form of memory involved in S.A.. The present experiments compared the effects of ibotenic acid lesions of MM in either sequential (successive trials separated by intertrial intervals ranging from 30 sec to 3 min) or delayed (2 forced trials followed by a test free trial given up to 6 hours) procedures using S.A., reinforced win-shift or win-stay protocols. Results showed that when tested with the win-shift rule, MM-lesioned mice still exhibited deficits as compared tc controls but with longer intervals than in spontaneous alternation. The use of the win-stay rule, however, produced no observable deficit in contrast to that seen in ther S.A. and win-shift protocols. These results suggest that MM lesions might impair primarily automatic form of memory.

494.27

SPATIAL BEHAVIOR OF NORMAL AND SEPTAL RATS ON ALTERNATE ROUTE MAZE PROBLEMS. T. Herrmann, B. Bolson* and B. Poucet Dep. of Psychol., U. of Guelph, Guelph, Ontario N1G2W1.

The behavior of septal lesioned and normal rats was compared on a variety of alternate route variations of the 3-table problem. Septal rats displayed impaired test trial behavior on all variations of the task. However, the route choice pattern did provide some insight into the nature of the septal deficit. Septal rats were able to distinguish and use a consistent route during both exploration and test trial phases. If available, the chosen route was usually the most direct between any two tables. When no direct route was available, septal rats took the boundary route, i.e the route which formed the peripheral edge of the apparatus In contrast, normal rats chose the most direct route only in the simplest configurations. In the more complex configurations, normal rats consistently chose the central but longer route which may have allowed delayed choice or single point reference orientation.

These results suggest that septal rats are able to store and use information about distances if not locations, and that normal rats are able to dissociate routes and locations according to several other variables.

494.28

OBJECT EXPLORATION, HABITUATION AND RESPONSE-TO-CHANGE IN RATS FOLLOWING FRONTAL CORTEX LESIONS, SEPTAL LESIONS, OR SCOPOLAMINE INJECTIONS. B. Poucet, C. Thinus-Blanc and M.-C. Buhot*. CNRS, Lab. of Functional Neurosc., Ulbis, 13402 Marseille cedex 9, France.

The possible involvment of the central cholinergic system in the exploratory behavior of objects in an open-field was studied in rats following a) medial frontal cortex lesions (FC), b) septal lesions (S), and c) i.p. scopolamine (1mg/kg) injections (SC). When compared with normal animals S rats 1) displayed a lower level of initial exploratory activity, 2) did not habituate, and 3) provided no evidence of reaction to the displacement of an object. FC rats were very similar to normal rats except for their response-to-change behavior which focused exclusively on the displaced object.

In a similar task using both a spatial and a non spatial change test, SC rats displayed a low level of initial exploratory activity and did not habituate over time in much the same manner as S rats. In addition, SC rats a) did not evidence reaction to either a spatial or a non spatial change and b) developed a stereotyped pattern of locomotor activity.

These results point to a) the central role of the septo-hippocampal cholinergic system in object exploration, and b) a non specific disruption by scopolamine of cholinergic related attentional processes.

494.29

STIMULATION OF THE LATERAL SEPTUM IS A MORE EFFECTIVE CS THAN STIMULATION OF THE MEDIAL SEPTUM DURING CLASSICAL CONDITIONING OF THE EYEBLINK RESPONSE.
B.J. Knowlton and R. F. Thompson. Department of Psychology, University of Southern California, Los Angeles, CA 90089.

Eight rabbits were trained in the classically conditioned eyeblink response procedure using stimulation of the septal nuclei as the conditioned stimuli (CS). Each rabbit was trained with both medial septal stimulation and lateral septal stimulation. Stimulation of the medial septum was a far less effective CS than stimulation of the lateral septum. This effect may be due to the different roles of these two nuclei in classical conditioning. Conditioning using lateral septal stimulation as a CS is dependent on the cerebellar interpositus nucleus, as is conditioning using peripheral and other brain stimulation CSs.

This research was supported by a National Science Foundation predoctoral fellowship to BJK and grants from the McKnight Foundation (22-1873-4988), the National Science Foundation (53-4873-6578), the Office of Naval Research (NOOO14-83) and the Sloan Foundation to RFT.

494.30

INSULAR PREFRONTAL CORTEX LESIONS PREVENT INCENTIVE CONTRAST EFFECTS IN THE RAT.
Ann Robertson and Andrê Laferrière*. Dept. of Psychology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E9.

The role of the anterior agranular insular cortex (AI) in mediating the over-response to unexpected shifts in the quality of food reinforcement (incentive contrast effect) was evaluated in two expts. In the first, rats with bilateral electrolytic lesions of the AI and sham-operated rats were trained to bar-press for either a highly preferred or less preferred liquid reward. Once performance had stabilized, the reinforcers were reversed. Control rats displayed significant contrast effects when their performance after the switch in rewards was compared to that of the rats receiving the same reward before the switch. However AI lesioned rats failed to show any contrast effect, even though they could exhibit sizeable preferences. In the second expt., the ability of AI lesioned rats to change their response rates as a function of variations in response contingencies was studied using a procedure which selectively reinforced responding or not responding. AI lesioned rats could alter their response rates to match changing schedule requirements as well and as fast as controls. This rules out motor perseveration as the reason for the lack of contrast in the first study. The results suggest that the AI is specifically involved in the response to novel changes in the quality of reward.

494.31

CONCURRENT LEARNING AND RETENTION TESTED WITH REVERSIBLE COOLING OF VENTROMEDIAL TEMPORAL CORTEX.
P. George, R. Cirillo*, Q. Chen* and J. Horel. Dept. of Psychology, Syracuse University and Dept. of Anatomy and Cell Biology, Health Science Center, Syracuse, NY 13210.

We have found a strip of cortex on the ventromedial temporal lobe (VMT) that is essential for performance of delayed match-to-sample, while the rest of the temporal cortex is not. It extends from posterior parahippocampal gyrus to anterior ventral inferotemporal cortex. Previously, we had found that small inferotemporal lesions impair learning but not recall and here we ask the same question of VMT. VMT was covered with a single cryode on each side of monkeys that were then trained on a concurrent learning and retention task. They were overtrained on a set of four object discriminations and these were presented concurrently with four new discriminations to measure normal learning and retention. The overlearned pairs were then presented together with four new discriminations while cooling VMT. The hypothesis was that with VMT suppressed, the animals would recall the overlearned discriminations but not learn the new discriminations; however, they were severely impaired both in learning and recall. This is the most severe retention deficit we have found with small temporal cortex lesions. (Supported by NINCDS grant NS18291).

494.32

PARAHIPPOCAMPAL GYRUS AFFERENT CORTICAL CONNECTIONS AS DEMONSTRATED BY RETROGRADELY LABELING CELLS WITH WGA-HRP.
C.L. Martin-Elkins* and J. Horel (SPON: J. Horel). Dept. of Anatomy, SUNY Health Science Center, Syracuse, NY 13210.

Inferotemporal cortex (IT) has been shown to play an important role in acquisition and retention of visual information. While it has generally been considered a single, homogenous visual area, recent studies have found anteroventral IT to be a distinct functional subdivision within IT. Injections of WGA-HRP in anteroventral IT, demonstrated a strong projection from the parahippocampal gyrus in area TF (Martin-Elkins, Soc. Neurosci. Abstr., 1987). Subsequently, cooling of this area was found to produce deficits on the DMS task similar to those found with anteroventral IT cooling. In an attempt to further examine the visual pathways into anteroventral IT, injections of WGA-HRP were placed in the parahippocampal gyrus (TF). Preliminary results show labelled cells in the parahippocampal gyrus, both anterior and posterior to the injection site, and in ventral IT. Similar to anteroventral IT, parahippocampal gyrus appears to receive little input from dorsal IT, since only a few, scattered labelled cells were located there. Posteriorly, cells have been mapped in the intraparietal sulcus, the rostral bank of the lunate sulcus, and the occipitotemporal gyrus. (Supported by NINCDS grant HS18291)

494.33

TEMPORO-PREFRONTAL INTERACTION IN RULE LEARNING BY MACAQUES.
J.A. Weinstein*, R.C. Saunders, and M. Mishkin (SPON: B. Turner). LN, NIMH, Bethesda, MD 20892.

Although combined amygdalo-hippocampal removals in macaques severely impair their performance on delayed nonmatching-to-sample (DNMS) when the delays between sample and choice exceed about 10 seconds, they can master the task with shorter delays. Such mastery cannot depend on the formation of specific visual discrimination habits, because (a) a different pair of objects is used on every trial and (b) within a trial, the reinforcement contingencies for responses to the sample object are inconsistent. To master the task in the absence of the limbic system, the animal must be able to learn a rule, which requires, in turn, (i) suppression of specific stimulus-response habits, (ii) abstraction of sameness and difference from specific stimulus quality with the aid of immediate memory, and (iii) formation of a stimulus/difference-response habit. We have now found that if inferior prefrontal lesions (which produce a moderate DNMS impairment by themselves) are added to amygdalo-hippocampal lesions, monkeys lose the ability to perform DNMS even when the delays are less than 10 seconds. This finding suggests that the inferior prefrontal cortex serves one or more of the processes described above needed for rule learning, and that it does so by mediating a complex set of interactions between the inferior temporal cortex and the neostriatum, with both of which the inferior prefrontal cortex is directly interconnected.

494.34

IMPAIRMENT OF SPATIAL, OLFACTORY, AND AUDITORY SERIAL REVERSAL IN AN ANIMAL MODEL OF HUMAN DIENCEPHALIC AMNESIA.
R.L. Knoth, R.G. Mair, S.A. Rabchenuk. Department of Psychology, University of New Hampshire, Durham NH 03824. P.J. Langlais. San Diego VAMC.

The post thiamine deficient (PTD) rat is an animal model of Wernicke-Korsakoff's disease, the most common cause of global diencephalic amnesia in humans. Behavioral testing of PTD rats has demonstrated learning and performance deficits on both aversively and appetitively motivated spatial tasks, including spatial delayed alternation and spatial delayed non-match to sample, but no deficit on spatial (left/right) and visual (light/dark) discrimination (Mair et al., Brain Res., in press; Knoth et al., Neurosci. Abstr., 13, 1127). The purpose of this experiment was to see the degree to which the learning and performance deficits extended to other sensory modalities.

A total of 16 PTD rats were compared to 24 controls on spatial, olfactory, and auditory serial reversal (SR). On spatial SR, experimental animals required consistently more trials than controls on initial learning and all subsequent reversals. On olfactory SR, experimental animals required significantly more trials on initial learning and completed less reversals overall. This same deficit was observed in auditory SR.

These results support the global nature of the learning and performance deficits observed in PTD rats.

494.35

ALTERED EXPLORATORY ACTIVITY IN AN ANIMAL MODEL OF DIENCEPHALIC AMNESIA. S.A. Rabchenuk*, R.G. Mair, & R.L. Knoth (SPON: E. Hagstrom). Department of Psychology, University of New Hampshire, Durham, NH 03824.

The post thiamine deficient (PTD) rat is an animal model of diencephalic amnesia, characterized by medial thalamic lesions and impaired performance on tasks measuring learning and memory. In this experiment, we videotaped open field activity of 16 PTD and 16 control animals during 3 daily 15 minute sessions. On day 1 and 2, animals were placed in an empty circular field and on day 3 a novel stimulus was placed in the center. Videotapes were coded in 1 minute intervals for line crossing, a measure of locomotor activity, and rearing, a behavior in which rats stand on their hindlegs and execute sniffing bouts and multiple shifts in head position.

Results showed that on days 1 and 2, PTD rats exhibited significantly more line crossings than controls. Both groups showed a decrease in the frequency of line crossing and rearing within each session. On day 3, the controls made more approaches to the novel object during the first 1 minute interval. However, controls made a rapid decrease in the frequency of these responses and PTD animals did not. This pattern of increased line crossing and decreased rearing was limited to rats with the lesion of the intralaminar thalamic nuclei typical of the PTD model.

MUSCLE: STRUCTURAL CHARACTERISTICS

495.1

FIBER TYPE COMPOSITION OF FATIGUE INTERMEDIATE MOTOR UNITS IN THE DIAPHRAGM. J.G. Enad* and G.C. Sieck. Dept. of Biomed. Eng., USC, LA, CA

The purpose of this study was to determine the fiber type composition of motor units (MU) in the cat diaphragm. MU's were classified as fast or slow (S) based on the sag test. Fatigue resistance (fatigue index, FI) was used to further subclassify fast units as FR (FI>0.75), FInt (0.25<FI<0.75), or FF (FI<0.25). MU fibers were identified using the glycogen depletion technique. Fibers were classified as type I or II based on ATPase activity. Subclassification of IIA and IIB fibers was based on ATPase activity after acid preincubation (pH 4.2 and 4.6). Fibers belonging to S units were uniformly composed of type I fibers. FR and FF units were composed exclusively of types IIA and IIB fibers respectively. FInt units showed a mixed fiber type compostion (both IIA and IIB). Those FInt units with FI<0.50 were comprised primarily of IIB fibers (80% vs 20% IIA), whereas FInt units with FI>0.50 were comprised primarily of IIA fibers (90% vs 10% IIB). We conclude that, unlike the homogeneous fiber type composition of S, FR, and FF MU's, FInt units are composed of a mixed population of type II fibers with proportions of IIA and IIB fibers related to unit fatigue resistance.

495.2

WHY ANIMALS HAVE DIFFERENT MUSCLE FIBER TYPES. L. C. Rome*, R. P. Funke*, R. McN. Alexander*, and G. Lutz*.(SPON: E. F. O'Connor). Dept. of Biology, Univ of Penn., Phila, PA 19104

It is assumed that animals have slow fibers because they are more efficient at low speed of locomotion, but need fast fibers because the slow ones cannot shorten fast enough to power rapid movements. To test these theories one must determine "where on the force-velocity curve" fibers are operating during locomotion (V/V_{max}; where V is the velocity at which the muscle is shortening during locomotion and V_{max} is the maximum velocity of shortening). V_{max} of carp slow red fibers = 4.65 lengths/s, that of fast white fibers = 12.9 lengths/s. During swimming at 20-40 cm/s (only the red fibers are active), V of red fibers = 0.7 to 1.7 lengths/s (V/V_{max} = 0.15-0.36 -- high power and efficiency.) At the same speeds, because of the different orientation of the fibers, the V of white muscle would = 0.2 - 0.4 lengths/s (V/V_{max} = 0.01 - 0.03 -- very low power and efficiency). To power the "startle response" (maximal movement), the red fibers would have to shorten at 20 length/s which far exceeds their V_{max}. The white fibers need shorten at only 4.85 lengths/s (V/V_{max} = 0.38 -- high power and efficiency). Even if the red muscle were placed in the orientation occupied by the white, it still couldn't shorten fast enough to power this movement.

We proved that animals need fast fibers to power maximal movements and provide strong evidence that slow fibers are more efficient at low speeds. Supported by MBL Summer Fellowship, Whitaker Foundation, NIH(AR38404) to LCR.

495.3

PREDICTION OF FIBER TYPES IN THE CAT QUADRICEPS MUSCLE. B.G. Samojla, L.L. Glenn, P.J. Rebeta*. *Department of Physiology, Ohio College of Podiatric Medicine, Cleveland, Ohio 44106-3082.*

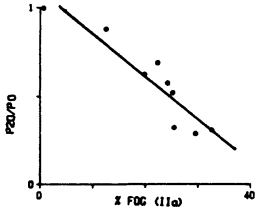
Neuromuscular compartments (NMC) have been identified in various muscles of the cat hindlimb including the quadriceps. The purpose of this study was to determine if the ratio of the tension at 20 Hz (P_{20}) vs the tension at 100 Hz (P_0) could predict the fiber type distribution in the nine quadriceps NMC.

For each NMC, single twitch tension, tension at 20 Hz and 100 Hz, and fatigue index was determined. The proportion of fiber types in each NMC was correlated to the contraction properties.

The average fiber type distribution (across all nine quadriceps NMCs) was SO = 35.8% (3.4 - 96.5), FOG = 22.4% (0.5 - 37.5), FG = 38.7% (2.2 - 63.6). The range of P_{20}/P_0 was 0.28 - 1.00 while the fatigue index ranged from 0.19 - 0.70.

The correlation coefficients of P_{20}/P_0 were -0.94 with FOG, 0.79 with SO, -0.58 with FG.

Our findings show that P_{20}/P_0 can accurately predict the percentage of FOG fibers in NMC of the cat quadriceps.



495.4

FIBER-TYPING BY ATPase HISTOCHEMISTRY OR MYOSIN IMMUNOHISTOCHEMISTRY : EFFECT OF DENERVATION ON MUSCLES OF C57BL/6J MICE. H.L. Davis and G. Desypris, Dept of Anatomy and School of P & OT, McGill U, Montreal, Canada, H3A 2B2; Dept of Physiology, U of Ottawa, Ottawa, Canada, K1H 5A3.

After unilateral hindlimb denervation of 2 wk mice for 4 wk or of 12 wk mice for 20 wk, sections of denervated (DN) and contralateral normal (NOR) extensor digitorum longus (EDL) and soleus (SOL) muscles were stained for ATPase (I,IIA and IIB fiber types) or immunohistochemically using monoclonal antibodies against fast (IIA or IIB) or slow (I) myosin heavy chain. Sections were examined for relative proportions of the various fiber types. Denervation had no effect on the total number of fibers but the proportions of fiber types were altered. Histochemically, DN muscles of young mice exhibited complete loss of type I fibers, whereas the proportion of those in muscles of older mice were unaffected ; for all mice there was marked dedifferentiation of type II fibers in DN EDL and SOL. Immunohistochemistry indicated very different results: fibers containing type I myosin increased in both DN EDL and SOL; in the latter they comprised nearly 100% compared to 33% in NOR controls. In DN EDL there were more fibers containing IIA and fewer with IIB myosin than NOR. These results indicate that histochemical and immunohistochemical techniques of fiber classification are not equivalent when examining plastic changes resulting from a perturbation such as denervation. Support: MRC and MDAC.

495.5

GENDER SPECIFIC MUSCLE FIBER AND STRENGTH ADAPTATIONS IN THE HUMAN BICEPS BRACHII.

S.E. Alway, J. Stray-Gunderson, W.J. Gonyea and W.H. Grumbt. Department of Cell Biology and Anatomy, UT Southwestern Medical Center, Dallas TX 75235

Isokinetic strength measures and computer tomographic scans were obtained on the elbow flexors of 7 elite male bodybuilders (MB) and 5 elite female bodybuilders (FB) to determine if the linear relationship between muscle CSA and strength is altered if strength is measured at high and low velocity contractions. A second purpose was to determine if male and female bodybuilders have similar ratios of strength/muscle CSA at high and low velocities of contraction. Absolute strength was significantly greater at all velocities of contractions in MB relative to FB. Strength/flexor CSA was, however, similar at 60° and 120°/s among subjects. Strength/flexor CSA was significantly lower at 180°, 240° and 300°/s in MB relative to low velocity contractions, however, this ratio was not reduced at high velocities in FB. Mean fiber cross-sectional area which was determined from biopsies of the biceps brachii was significantly greater in MB (8794.1 ± 1059.7 μm^2) relative to FB (5023.4 ± 772.7 μm^2). Mean fiber area correlated strongly to strength/biceps CSA at low (r=0.8) but not high velocities (r=0.4) of contraction. A large inter-subject range in fiber number estimated from the biceps (603,252-187,299), likely prevented a positive correlation between strength and fiber number at any velocity of contraction (r=0.1) The results indicate that adaptations of strength/CSA in bodybuilders are gender specific.

495.6

RECORDING HUMAN SINGLE MOTOR UNIT PROPERTIES: A NEW METHOD.

R.S. Johansson, G. Westling*, C. K. Thomas and B. Bigland-Ritchie. Umeå University, Sweden & J. B. Pierce Foundation, New Haven, CT 06519.

The contractile properties of individual motor units of human thenar muscles were examined by a new method derived from the technique of microneurography and analogous to that used in animal studies. Forces of both flexion and abduction were recorded simultaneously from the thumb, together with EMG from both the proximal and distal muscle surfaces, while a tungsten microelectrode was used to stimulate single motor axons in the median nerve above the elbow. When the stimulus current was increased from zero, unitary activity was accepted if: a) no force or EMG responses were seen below a critical stimulus intensity; b) signals then appeared simultaneously, and remained unchanged over a wide range, usually 2-4 μA, before any graded increments appeared; c) X/Y plots of the abduction and flexion forces showed a characteristic force vector for each unit. These criteria were satisfied repeatedly for 45 units from 12 subjects. Unit responses often remained stable for up to 1hr while applying different stimulation protocols, including 1s bursts of constant frequency (1-100Hz), pulse intervals varied to optimize force generation, and standard fatigue tests. Respiratory and circulatory baseline fluctuations were minimized by triggering each stimulus packet from the heart beat, and electronically re-setting the force baseline to zero just prior to each response. Contractile speeds, force/ frequency curves and axon conduction velocities were measured before and after fatigue. Burke fatigue indices were calculated, together with each unit's characteristic angle of pull within the muscle.

Supported by USPHS and the Swedish Medical Research Council.

495.7

TWITCH PROPERTIES OF SINGLE HUMAN MOTOR UNITS.

C. K. Thomas, R.S. Johansson, G. Westling*, and B. Bigland-Ritchie. John B. Pierce Foundation, New Haven, CT. 06519 and Umeå University, Sweden.

Twitches properties of thenar human motor units examined by stimulating single motor axons (Johansson et al., 1988 above), were compared with those measured previously by spike-triggered averaging (Thomas et al., J. Neurophysiol. 57:311, 1987). Twitch amplitudes of 45 units from 12 subjects, measured at the start of each experiment, ranged from 2.9 to 34.0 mN (mean ±SD; 11.4 ± 8.1 mN). However, less than 25% had amplitudes greater than 15.6mN. The corresponding twitch contraction and half-relaxation times had a near Gausian distribution (50.4 ± 9.2 & 59.9 ± 18.5 ms respectively). Axon conduction velocities were 45.9±5.4 m/s. These units generated force at angles between -47° to 124° relative to thumb flexion. Following other tests, the twitches potentiated, some by up to 4 fold. Twitch contraction and half-relaxation times also increased. The distribution of twitch amplitudes, both before and after potentiation, was similar to that found using spike-triggered averaging from abductor pollicis brevis muscles. However, using the latter method, higher values were also reported, some with amplitudes twice those of the largest measured in the present study. These values may be due to inevitable synchronization between unit discharge rates and some twitch potentiation during spike-triggered averaging.

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495.8

CLASSIFICATION OF HUMAN MOTOR UNITS.

B. Bigland-Ritchie, C. K. Thomas, G. Westling*, and R.S. Johansson. John B. Pierce Foundation, New Haven, CT. 06519 and Umeå University, Sweden.

To determine how far human motor unit contractile properties corresponded with those of other mammals, twitch and tetanic responses of the thenar muscle were examined while stimulating individual motor axons (Johansson et al., 1988; Thomas et al., 1988, above). Twitch amplitudes were not correlated with their corresponding contraction or half-relaxation times, nor their axon conduction velocities (45 units). "Sag" was not tested systematically, but none was seen when stimulating at 8, 10, 15 or 20Hz. Burke fatigue indices, measured from 25 units, varied continuously from 0.43 -1.28. Only 36% of the units showed < 25% force decline. During the Burke fatigue test relaxation from the 40Hz responses generally slowed markedly. Thus, using criteria normally applied to animal units, there was no indication that these human motor units can be separated into distinct types, based solely on differences between their contractile properties. More data are required about the histochemical composition of the human thenar muscles (63% type I, 37% type II; Johnson et al., J. Neurol. Sci.18:111, 1973) before conclusions can be drawn as to differences between animal and human motor unit properties or their relation to histochemical typing.

Supported by USPHS grant NS 14657 and HL 300062, and the Swedish Medical Research Council.

495.9

THE DISTRIBUTION AND ULTRASTRUCTURE OF INTRAFUSAL FIBERS IN THE CAT MASSETER MUSCLE.

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To provide initial data regarding the distribution and ultrastructural properties of masticatory muscle spindles, the superficial and deep masseter muscles were dissected from adult cats immediately after perfusion-fixation. Muscles were divided into smaller pieces which were then serially cut into 1 mm thick blocks. Blocks were processed for electron microscopic (EM) study. Neuromuscular spindles identified on 1 μm thick sections were thin sectioned for EM analysis. A total of 65 spindles were identified in 4 muscles. Most of the spindles were located in intermediate to deep portions of the masseter muscle near its attachments to the zygomatic bone as described by Lund et al. (Neuroscience 3:259). Single spindles and spindle pairs occurred most commonly, although larger complexes were sometimes observed. The number of intrafusal fibers/spindle ranged from 1-10 (x=4.2) in polar regions. In the equatorial region, fibers were easily classified as nuclear bag (NB) or nuclear chain (NC). Although the NB fibers were typically larger than NC fibers, there was overlap with respect to the diameter of these two types of intrafusal fibers. In juxtaequatorial regions, the M-line of the sarcomeres, prominent in NC fibers, were either totally absent or represented as pale double lines in NB fibers. Chain fibers possessed numerous large mitochondria in juxtaequatorial and polar regions. Bag fibers could be divided into type 1, which had sparsely distributed thinner mitochondria, or type 2, which had numerous larger mitochondria. Masseter spindles were generally comparable in structure to spindles described in limb muscles (Kucera, Histochemistry 79:457). However, there seemed to be a larger percentage of multiple bag spindles in the masseter. The diversity in spindle intrafusal fiber composition could account, in part, for differences observed in the response properties of spindle primary afferents (Inoue et al., Exp. Neurol. 74:548). Supported by NIDR DE06027.

495.10

FIBER ARCHITECTURE OF LONG, PARALLEL-FIBERED MUSCLES IN THE CAT HINDLIMB: EVIDENCE OF INTRAMUSCULAR TAPERED ENDINGS.

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Available evidence indicates that the long, parallel-fibered muscles, sartorius (SA), tenuissimus (TEN), and the distal head of semitendinosus (St) are comprised of relatively short (~ 3.0 cm) interdigitating muscle fibers arranged in staggered longitudinal series (Loeb et al. J. Morph. 191: 1987). The presence of very small (cross-sectional areas ≤ 300 um^2) muscle fibers (VSMFs) in TEN (Lev-Tov et al. J. Neurophysiol. 59: 1988) supports other evidence (Loeb et al. ibid) that muscle fibers in muscles with this architectural arrangement have tapered intramuscular endings. If VSMFs reflect tapered endings, they should occur within all fiber types.

In this study, the incidence of VSMFs within each muscle fiber type was determined in SA, St, and TEN and compared to similar data obtained in selected pinnate muscles (medial gastrocnemius (MG), tibialis anterior (TA) and tensor fascia latae (TFL)). Fiber areas were digitized in serial cross-sections (15 um) that had been stained so as to classify muscle fibers according to their histochemical properties (SO, FOG, FG). In some experiments, prolonged stimulation of single SA or TEN motor axons in ventral root filaments was used to deplete their muscle fibers of glycogen.

With the exception of TEN, pinnate and parallel-fibered muscles did not differ in the upper limits of their fiber size distributions, but they were significantly different in the areas of their smallest fibers. Thus far, VSMFs were found in FOG and SO fibers in TFL and in all muscle fiber types in SA and TEN. The proportion of VSMFs in depleted fast twitch muscle units in SA was 8-11% and 9-53% in TEN. These data indicate that VSMFs reflect an architectural feature in parallel-fibered muscles that is independent of the normal size-histochemical relationships.

495.11

AN ANATOMICAL STUDY OF MOTOR END PLATES IN A COMPARTMENTALIZED MUSCLE. O.I. Weeks. Dept. of Biological Sciences. Florida International University., Miami, FL 33139

Previous studies (English & Letbetter '82, English & Weeks '84, Weeks & English '85, '87) have suggested that neuromuscular compartmentalization is a basic organizational feature in some skeletal muscles. To determine whether neuromuscular compartments can be reestablished following injury, the mouse LG muscle has been adopted as a study model. A baseline for compartmentalization in this muscle is also being established. The present study analyses the quantitative and qualitative profile of motor end plates (meps) in the mouse LG muscle using acethylthiocholine, bromoindigo and silver, or zinc-iodide osmium staining methods. Results show that meps are distributed on equatorial zones of both superficial and deep muscle fibers in each compartment. Type b & c meps (classification of Korneliussen & Waerhaug '73) are found throughout each compartment. Type b meps tend to be more commonly found in distal and ventral compartments which consist of more oxidative muscle fibers. Additionally, meps were also observed in various stages of degeneration. The frequency with which degeneration occured tended to be associated with collateral branching and did not appear to be compartment specific. This definition of the basic anatomy of meps is an attempt to further characterize LG neuromuscular compartments and make it more convenient for studying compartmental reinnervation.

495.12

ARCHITECTURE AND PERIPHERAL INNERVATION OF A MULTI-FUNCTION NECK MUSCLE. R.J. Callister and E.H. Peterson. Dept. Zoological & Biomed. Sci. & College of Osteopathic Med. Ohio Univ., Athens, Ohio 45701.

As part of a study aimed at understanding neuromuscular control of movement parameters we are examining the construction and innervation of the head retractor muscle RCCQ in a turtle, P.scripta. This strap muscle is used in the characteristic turtle startle response, in feeding, and in head steering during locomotion. Thus RCCQ mediates movements requiring widely different speed, force, and geometry. We used single fiber dissections of gold chloride stained muscles to examine muscle architecture. We also visualized: intramuscular territories of segmental nerves to RCCQ in muscle wholemounts using sudan black staining, motor end plate (MEP) zones in muscle wholemounts and single fibers with cholinesterase, and MEP morphology by anterograde transport of HRP applied to muscle nerves.

Individual fibers vary widely in maximum diameter (20-80um in 11cm turtles); they do not form clear size classes. Most of these presumably correspond to the fast fiber types that dominate RCCQ (Fg-60%, FOG-30%; Callister et al., '87, Soc. Neurosci. Abs. 13:1217). Fibers also vary in length (20-100% of total muscle length). There is a significant positive correlation between fiber diameter and length; approximately 70% of the largest fibers (>50um), compared with 16% of other fibers, span the full muscle length (approx 6cm). Shorter fibers are arranged in series throughout the muscle; many taper over long distances and are bound by connective tissue to other tapered fibers as well as to fibers which span the full muscle. Three of the four bellies are innervated by 2-3 segmental nerves each, whose intramuscular territories show little overlap. Single fibers bear (2-7) focal, MEP zones at 2-16mm intervals. Thus long single fibers have multi-segmental, and probably multi-neuronal, innervation. Motor axons contact the sarcolemma in 1-4 longitudinally oriented, varicose strips. There is a significant positive correlation between muscle fiber diameter and both the number and length of contacting varicosities, suggesting that larger fibers may bear greater synaptic areas.

Our data indicate that RCCQ contains >1 fiber population, and that these are structurally (as well as histochemically) specialized for different roles in head movement control. The shortest fibers, with their small diameters, tapered profiles, and series construction are poorly suited to mediate the startle response. In contrast, the largest diameter fibers exhibit multiple adaptations for high speed /force output. They probably belong to >1 motor unit, but how these motor units are coordinated is not known. (Supported by NIH grant RO1NS23498)

495.13

ULTRASTRUCTURAL CHARACTERIZATION OF WING RETRACTION MUSCULATURE IN THE PTEROPOD MOLLUSC CLIONE LIMACINA. Z. Huang*, M. Titus* and R.A. Satterlie (SPON: J. Harris). Dept. of Zoology, Arizona State Univ., Tempe, AZ 85287

Tactile stimulation of the wing-like parapodia of the pteropod mollusc Clione limacina can trigger a wing retraction reflex, during which the wing is deflated and pulled into the body. Full retraction is achieved within 2-3 seconds and the wings usually remain retracted for 20-40 seconds. The magnitude of retraction is a graded function of the stimulus intensity.

Three groups of smooth muscles involved in wing retraction are found in the wing haemocoeal: the transverse muscles, longitudinal muscles and dorsoventral muscles. Furthermore, two subtypes of muscle cells were identified. The first type (type A) appears in all three groups of muscles and forms a well organized lattice-like structure. The second type (type B), being the major component of transverse muscles, run only one dimensionally.

Quantitative ultrastructural comparisons between the two types of smooth muscles suggest that type A cells are able to contract and relax more quickly with low endurance while type B cells are capable of generating stronger contractions with higher endurance and slower relaxation speed. The role of these cell types in wing deflation and retraction is now under investigation.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: POSTURE AND MOVEMENT VIII

496.1

ADAPTATION TO TELESTEREOSCOPIC VIEWING MEASURED BY ONE-HANDED BALL-CATCHING PERFORMANCE. S.J. Judge and C.M. Bradford*. University Laboratory of Physiology, Parks Road Oxford, England OX1 3PT.

A one-handed ball-catching task was used to study the disturbance of depth judgement induced by telestereoscopic viewing (i.e. viewing with effective interocular separation increased, in this case by a factor of approximately 2.3), the recovery of performance with experience in the telestereoscope, and the errors that subsequently arose when the telestereoscope was removed. Performance was videotaped. On first wearing the telestereoscope, subjects closed the hand when the ball was approximately twice as far away from the eyes as the hand. After less than 20 trials in the telestereoscope, subjects were closing the hand at approximately the correct time and place, although rather more trials were needed for ball-catching performance to recover to normal. When the telestereoscope was removed there was an after-effect with reaching errors in the opposite direction. A number of possible explanations of these data are considered.

496.2

AN EXAMINATION OF THE ROLE OF CENTRAL AND PERIPHERAL VISION IN PREHENSION. *B. Sivak and C.L. MacKenzie. Department of Kinesiology, University of Waterloo, Waterloo, Ontario Canada, N2L 3G1.*

During prehension, visual information is provided by both central and peripheral visual fields. In our study subjects reached for and grasped a dowel 2.5 cm in diameter using either peripheral vision only or central vision only. A three dimensional movement analysis yielded information about reach and grasp components during prehension. For the reach component with peripheral vision only, the proportion of movement time spent after peak velocity, acceleration and deceleration did not change compared to normal vision. However, movement time was longer and overall speed was slower with peripheral vision only than normal vision. The grasp component was affected. For the reach component with central vision only, subjects reached a lower overall speed, movement time was longer and the proportion of movement time spent from peak acceleration, velocity and deceleration was longer for central vision only than for normal vision. The grasp component was not affected with central vision only compared to normal vision. Results suggest that central and peripheral vision provide specific information to the organization of prehension.

496.3

THE EFFECT OF OBJECT SIZE AND TYPE OF GRASP ON PREHENSION. *C.L. MacKenzie and B. Sivak. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.*

A recent study by Marteniuk and Athenes (1986) has shown that a high correlation exists between the size of the opening between the index finger and thumb and the object to be grasped. The present study examined how visual information from varying size dowels integrates with the motor system when prehension requires either a collective or independent finger grasp. A three dimensional movement analysis yielded information about the grasp component during prehension movements. Subjects were required to reach for and grasp dowels varying in size from 1 cm to 7 cm in 1.5 cm increments using either a collective or an independent finger grasp. Analysis of the peak aperture revealed a grasp type by dowel interaction. Consistent with the Marteniuk study, peak aperture increased systematically for the 5 different dowel sizes when an independent grasp was used. However, although peak aperture was smaller for the smallest dowel and largest for the largest dowel size when a collective grasp was used, the increase was not systematic for all dowel sizes. This suggests that the visuo-motor integration process may be different for collective and independent grasps.

496.4

BEHAVIORAL & COMPUTATIONAL MODELLING USING BALLPARK MODELLING FOR STUDYING HUMAN PREHENSION, A.Iberall, Dept. of Computer Sci., Univ. of Southern Calif., Los Angeles, CA. 90089 and C.L. Mackenzie, Dept. of Kinesiology, Univ. of Waterloo, Ontario, Canada N2L 3G1.

Wing et al [JMB,1986] quantified prehensile movements, arguing for a differential contribution of thumb & fingers. Arbib et al [EBRS,1985] use schema theory to model these movements: a schema gets the wrist into the right'ballpark' of the location by adding a small delta to the goal location. Another one sets up a hand posture consisting of virtual fingers (VFs), again within some ballpark of the final posture. Once positioned, other schemas adjust these parameters, subtracting small deltas to ensure contact.

We performed a pilot study of a subject reaching in the sagittal plane for a horizontal dowel. At peak aperture the wrist was always 20mm of the value it would have at time of contact, and its orientation within 0.06 radians. These results indicate that the wrist was always in some ballpark at peak aperture. The orientation and length of VF1 were smaller at peak aperture than at contact (within 6mm and 0.06 radians). The orientation and length of VF2 was more variable. This supports Wing et al that the thumb seems to be more constrained.

We simulated these schemas using an Amari-Arbib neural net. Stronger weights were needed on VF1,due to the kinematic constraints. These simulation results support the behavioral results for ballpark modelling.

496.5

A KINEMATIC ANALYSIS OF PREHENSION IN APRAXIA. J.L. Charlton,*E.A. Roy, R.G. Marteniuk and C.L. MacKenzie. University of Waterloo, Ontario N2L3G1

The disorder of apraxia is primarily manifest in tasks demanding complex coordination and hand posture and orientation (Roy et al., 1986; Haaland et al., 1980). While difficulties arise when pantomiming object use, more successful performance is observed with the appropriate context of actual objects. This study examined the kinematic characteristics of apraxics' errors in reaching, prehension and object manipulations.

Coordination complexity and context were manipulated in 5 experimental conditions: 'reach'; 'reach and orient' the hand; 'reach, orient and grasp'; 'reach, orient, grasp and stir' with a dowel and imaginary cup; and with a real spoon and cup. Two apraxics and 3 non-brain-damaged control subjects participated in the study. The WATSMART system provided 3-dimensional data describing movements in terms of wrist resultant velocity and acceleration profiles and aperture profiles.

Analysis of wrist resultant velocity profiles indicated that apraxics exhibited smaller peak speeds and longer time in acceleration for all conditions. Furthermore, apraxics made more frequent adjustments of acceleration and deceleration than control subjects in Conditions 1 to 4, in which complexity was manipulated, suggesting a feedback guidance strategy. Apraxics also adopted a notably wider maximum aperture and spent longer percent time in closure, suggesting a compensatory strategy for spatial errors in guiding the hand to the object. When context was present, apraxics' wrist acceleration profiles and aperture profiles were more similar to control subjects. Context thus appears to be critical for apraxics' efficient performance. Results are discussed in terms of conceptual and production disorders of an action control system.

496.6

EFFECT OF INSTRUCTIONS ON COMPENSATORY ADJUSTMENTS DURING PRECISION GRIP.

C.J. Winstein, J.H. Abbs, and D.E. Petashnick*. Speech Motor Control Laboratories, Waisman Center, Univ. Wisconsin, Madison, WI 53706.

Successful grasp, lift, and manipulation of objects requires modulation of neuromuscular outputs by cutaneous and muscle mechanoreceptors in relation to motor set and task demands. Previous work has shown compensatory adjustments in grip force and associated muscle activity 60-80 ms following unanticipated object slips (Westling & Johansson, 1984; 1987). In this study, the effects of motor set on these grip adjustments were evaluated in four subjects. Unexpected vertical load perturbations were delivered to an object held between the thumb and index finger. Two instructional variations ("hold", "let-go") were combined with two load conditions (load, unload) resulting in four experimental conditions. Overall, a 15% increase in grip force (.6 N) was observed to loads and an 8% reduction (.3 N) to unloads. Average latency of these initial compensatory grip force changes was 55-85 ms. Several EMG changes accompanied the initial grip force adjustments, including increased and decreased activity to load and unload, respectively, in both adductor pollicis (50-60 ms latency) and extensor carpi radialis longus (12-30 ms latency). In addition, increased abductor pollicis brevis activity (50-60 ms) was associated with the unloads. Characteristics of these initial responses did not appear to be affected by the instructions. However, secondary changes associated with the "let-go" instruction were evident as early as 160 ms after perturbation in grip force, and as early as 135 ms in adductor and abductor pollicis activity. These secondary adjustments were thus influenced by motor set. In contrast to earlier work with long-latency muscle afferent responses, these findings suggest that initial compensatory grip adjustments of comparable latency may be unmodifiable by instructional variations. (Supported by NIH grants NS-13274 and HD-03352.)

496.7

TRANSITION OF PHYSIOLOGICAL TREMOR OF THE FINGER INTO FATIGUE TREMOR. S.S. Palmer and S.M. Bane* (SPON: G. Jackson). Depts. of Kinesiol., and Physiol. & Biophys., and Neur. & Behav. Biol. Program, Univ. of Illinois at Urbana-Champaign, IL 61801.

To resolve conflicting reports from different laboratories in regard to the transition of tremor frequencies and amplitudes under conditions of prolonged submaximal contractions, human subjects (6 female, 6 male) were asked to elevate the middle finger of their nondominant hand 1½" above the table top on which their hand was placed for 45-60 min. A lightweight accelerometer was taped to the finger to record tremor. EMG was recorded from extensor digitorum. Data stored on a tape recorder was analyzed using a spectral analysis program and an IBM PC/AT. Stretch reflexes were tested before and after the task by averaging 20 trials of rectified EMG.

All twelve subjects showed enhanced 8-12 Hz physiological tremor after 15-35 min, ranging from 2-115 ($\bar{x}$=28) times the original amplitude. Three subjects showed a progressive increase from 15-35 min, but even in these subjects variability predominated over clear progression. In 10 subjects measured, stretch reflex components 10-50 ms after stretch were decreased in amplitude after the task by 18-76% ($\bar{x}$=46%). 7/12 subjects showed a sudden onset of a 4-6 Hz tremor, but it was never both maintained and greater than the 8-12 Hz tremor. All humans probably experience greater physiological tremor with fatigue, but their susceptibility to a 4-6 Hz tremor varies.

496.8

COMPLEX OSCILLATIONS IN A HUMAN MOTOR SYSTEM. Beuter, A., Larocque, D.*, Glass, L.*. Univ. du Québec à Montréal and McGill University.

Experiments were performed to investigate the oscillations arising in a human motor system with delayed visual feedback. Eight subjects were instructed to maintain a constant finger position relative to a stationary baseline. The finger displacement was measured using a micro-displacement transducer connected to the index finger, and was displayed on an oscilloscope. Time delays between 40 and 1500 msec were inserted in the visual feedback loop for 100 sec. Results show that as the time delays increase irregular rhythms appear with short intermittent periods of regular oscillations. These regular low frequency oscillations have an amplitude that increases with the time delays and a period that is consistently about 2 to 4 times the time delay. Fast Fourier Transforms show a peak between 8 an 12 Hz corresponding to physiological tremor in half the subjects. No systematic variations in the FFT for the 2 to 15 Hz range were observed as time delay increased. In the 0 to 2 Hz range the FFT show a consistent increase in power with the time delay. These results indicate that under the conditions of this experiment, tremor is not affected by time delays in the visuo-motor system and that time delays in the feedback loops of motor control systems give rise to complex ocillatory behavior.

496.9

ABBERANT GRASP FORCE REGULATION AND DECREMENTS IN MANUAL FUNCTION AS A CONSEQUENCE OF REDUCED HAND SENSORY FUNCTIONING IN THE AGED. K.J. Cole and K. Grimes. Dept. Exercise Science, Univ. of Iowa, Iowa City, Iowa 52242.

Impairments of manual dexterity are commonly observed in the elderly without other signs of disease. Geriatric populations also demonstrate substantially reduced densities of cutaneous mechanoreceptors in the hands, particularly Meissner's corpuscles. Given recent demonstration of cutaneous sensory contributions to grasp force control during precision grasp (Johansson, R., Westling, G. Exp. Brain Res., 56: 550-564, 1984), it appears reasonable to hypothesize that impairments of manual dexterity in the aged are related to reduced hand sensibility. Thresholds for 2 point and moving 2 point discrimination at the finger pulps were obtained from elderly and young adults. "Manual dexterity" was assessed using common functional tests, such as the time needed to flip a series of playing cards that were lying flat on a table surface. Grasp forces during lifting of a test object with a precision grip were measured in the manner of Johansson and Westling (ibid). The elderly group showed significantly elevated tactile thresholds and increased times in the dexterity tasks. Grip forces were increased over control levels and were incompletely adapted with changes in the slipperiness of the test object. Dexterity and grip force control were related to tactile thresholds across both groups, rather than with age, per se.

496.10

ADAPTIVE VISUAL-MOTOR MODEL FOR CONTROL OF BEHAVIORAL SEQUENCES M. Kuperstein, Neurogen, 325 Harvard St. suite 211, Brookline, MA 02146

In the infant development of grasping behavior, there is an initial period of sequential groping to eventually grasp a stationary object. As the infant gains sufficient accuracy, grasping is typically optimized to one reach. How does the brain learn about the mistakes from the sequential groping experience and incorporate it into accurate control of grasping that is generalized throughout the volume of space? I will show an implemented neural model that suggests an answer.

Studies by Held, Hein and others in the last two decades have shown that, in the kitten, visually guided behavior develops only when changes in visual stimulation are systematically related to self-produced movement. This work extends these studies by hypothesizing that a *sense of space* emerges out of the correlation between object sensation and object manipulation.

The general strategy for accomplishing a representation for *space* is the sensory-motor neural circular reaction. In this reaction, motor activity for the entire range of grasping postures of an object are generated one at a time by some activating source. During each posture the two eyes see the 2-D projection of the 3-D object. Visual map activity is then correlated with whatever motor map activity was used to grasp the object. The correlation occurs from the changes of synaptic weights between visual inputs and motor outputs. After the correlation is learned, any object that is seen can trigger the visual maps to activate the motor map for the intended grasping posture of that object.

There are two key aspects to the current model called INFANT (Interacting Networks Functioning on Adaptive Neural Topographies): 1) A distributive, topographic architecture is used to combine the visual activity from an object, from both eyes and interface it with motor outputs. 2) Learning is achieved by incrementally modifying the distributions of input weights to the target map over single or sequential performance trials. On any given trial, only the weights of the active inputs in the trial are changed.

The neural model has been implemented into a working robot system with stereo cameras. The implementation learns to accurately grasp an object anywhere in space with one reach from the experience of sequential groping.

496.11

MECHANISMS OF HUMAN HEAD STABILIZATION DURING RANDOM SINUSOIDAL ROTATIONS. E.A. Keshner and B.W. Peterson. Dept. of Physiology, Northwestern University Medical School & Sensory-Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611.

Reflex, voluntary and passive biomechanical mechanisms are available to produce stability of the head-neck motor system. Guitton et al. (1986) found that longer latency, voluntary mechanisms dominated stabilization at frequencies lower than 1 Hz, but suggested that reflexes and biomechanics might be important at higher frequencies. To test this, we recorded chair and head velocities, and surface EMG's from splenius and sternocleidomastoid as seated subjects were rotated about the vertical axis. The random sum-of-sines stimulus had frequencies ranging from 0.185 to 4.18 Hz which should be high enough to elicit vestibulocollic (VCR) and cervicocollic (CCR) responses. Gain of head velocity and EMG responses were calculated using a best fit sinusoid. Four test conditions varied the relative influence of attentional processes and visual, vestibular, and proprioceptive feedback. EMG records revealed modulation of neck muscle activity in all 4 conditions. When subjects attempted to stabilize the head both with and without visual feedback, head stabilization in space was excellent at low frequencies and reached a resonant peak at about 2 Hz. When subjects performed mental arithmetic (MA), head stability was poor at low frequencies, but approached the other conditions above 1 Hz. Plotting head relative to trunk revealed a transition from neurally-dominated to inertially-dominated head stabilization at about 2 Hz. EMG activity plotted relative to head position in space, exhibited second-order lead behavior very much like that produced by the VCR in the cat. During MA, EMG activity increased with increasing head motion at higher frequencies, indicating that VCR and CCR contribute to stabilization. When actively stabilizing, subjects produced additional EMG output relative to head stabilization at lower frequencies, where EMG led head motion as expected. At high frequencies, lead was less, suggesting that VCR and CCR were responding to, and presumably damping inertially generated head movements.
Supported by grant NS22490.

496.12

TEMPORAL CHARACTERISTICS OF NECK MUSCLE EMG OF TRAINED CATS IN VISUAL ORIENTATION. M.-F. Decostre*, M. Crommelinck* and A. Roucoux. Lab. of Neurophysiology, Univ. of Louvain Sch. of Med., B-1200 Brussels, Belgium.

The distribution of EMG activity of different neck muscles in relation with orienting head movements has already been studied in the alert trained cat (Roucoux, A. et al. Soc. Neurosci. Abstr., vol. 11, p. 83, 1985). It has been shown that, on basis of the intensity of its global discharge, a preferential orientation could be attributed to each muscle: this orientation however covers a relatively wide angle with the consequence that many muscles contribute to a given movement. The purpose of this study was to examine the timing of the discharge of the different muscles as a function of the direction of the head movement. The latency, i.e. the delay between the burst and movement onsets, progressively increases as the movement direction diverges from the preferential orientation of the muscle: from a negative value of about 60ms (the burst precedes the movement) to a positive 70ms (the burst occurs during the movement). Some muscles show a late activity, occurring around the midcourse of downward movements. They may show inhibitions whose latency and duration are related to the direction of the movement.

In conclusion, the head motor system controls the direction and amplitude parameters not only by selectively activating the appropriate muscles but also by sequencing their activity to start, control the trajectory and stop the movement.

496.13

EYE, HEAD AND HAND COORDINATION WHILE GRASPING AND POINTING. H. Carnahan*, and R.G. Marteniuk. Dept. of Kinesiology, Univ. Of Waterloo, Ontario, Canada, N2L 3G1.

The sequential coordination of eye, head and hand movements under various task demands was studied. Six subjects were instructed to grasp and lift a wooden disk located on a table top in two different locations. In a second study six different subjects pointed as fast and accurately as possible to lights located at three locations. The initiation and termination of lateral eye movements were measured by an EOG and the WATSMART 3-D system was used to monitor the movements of the head and hand. Results from the first experiment showed that the head started to move an average of 75 ms before the eyes and 87 ms before the hand. In the second experiment relatively large variability masked any differences between head and eye start. The hand however, started last. The variability in this experiment appeared to be due to different strategies employed by the subject. Evidence is presented that on trials where the subject emphasized accuracy, the eyes moved first, and on trials where speed was emphasized the head moved first. These results are discussed in terms of planning and execution processes in multimovement systems.

496.14

POSTURAL POSITIONS YIELDING ALIGNED EIGENVECTORS OF COORDINATE FRAMES INTRINSIC TO VESTIBULAR AND HEAD-NECK MUSCLE SYSTEMS IN HUMAN. *A. Berthoz, M. Benamou* (Lab. Neurosensorielle, CNRS and Hopital Cochin, Paris, 75006, France) and *A.J. Pellionisz* (Dept. of Physiol. New York Univ. Med. Ctr. 10016) (SPON:J.I. Simpson)

Qualitative characterization (eg. depiction) of complex sensorimotor phenomena such as *posture* has been widely used, for its intuitive power, since ancient times. However, a *mathematical* encapsulation of what posture represents has been difficult because of a scarcity of concepts and formalisms suitable for integration and synthesis.

We propose an approach *to use multidimensional computer modeling techniques for a reconceptualization of posture in terms of functional geometry.* Given the great number of degrees of freedom which are to be controlled in maintaining a posture, we wish to identify constraints that CNS may be using in order to match systems of sensory information and motor command. The approach is based on models using *general coordinate systems that are intrinsic to the CNS.* Such functional coordinate axes can be established even in case of moving multiarticulate skeletomuscular systems with distributed centers of rotation. Using graphics-based computer anatomy, a preliminary model of human head-neck system has been developed, enabling us to calculate Eigenvectors of the neck-musculature and to compare them with the cardinal axes of the frame of vestibular canals. Initial results show that *the vestibular sensory and neck-muscle motor frames and their Eigenvectors rotate in different directions during head-pitch,* and thus in *two* distinct pitch-positions they are aligned. While models provide quantitative predictions for postural positions in which sensory and motor geometries match, experimentation will determine their accuracy. Maintaining such postural positions may be of advantage since co- and contravariant expressions may be identical and thus the sensorimotor transfer minimizes errors. -Support: CNRS & NS 22999-

496.15

TOPOGRAPHIC MAPPING OF PRESACCADIC BRAIN ELECTRICAL ACTIVITY IN HUMANS: SUPPORT FOR THE DUAL PREMOTOR SYSTEMS HYPOTHESIS. G. Goldberg and M. Moster*. Electrodiagnostic Center, Moss Rehabilitation Hospital and Temple University School of Medicine, Philadelphia, PA. 19141.

Voluntary saccadic eye movements are preceded by a series of event-related potential (ERP) components. The presaccadic negativity (PSN) beginning 500 to 800 ms before the saccade is analogous to the Bereitschaftspotential recorded in advance of finger movement and may reflect activation of the supplementary motor area (SMA--Deecke L et al, *Human Neurobiol* 4:143-154, 1985). A brief, large amplitude component called the spike potential (SP) immediately precedes the saccade. Cortical unit and microstimulation studies in monkeys (Schlag J & Schlag-Rey M, *J Neurophysiol* 57:179-200, 1987) and regional cerebral blood flow (rCBF) studies in humans (Fox PT et al, *J Neurophysiol* 54: 348-369, 1985) demonstrate a saccade-related cortical region rostral to SMA on the dorsomedial surface of the frontal lobe. Activational and microstimulation properties distinguish this "supplementary eye field" (SEF) from the classical arcuate frontal eye field. Guided by predictions from the **dual premotor systems hypothesis** (Goldberg G, *Behavioral and Brain Sciences* 8:567-615, 1985; 10:323-329, 1987), the dependence of the topography of presaccadic ERPs on behavioral context was examined in six normal human subjects. The degree to which timing and direction of saccades were exogenously constrained by visual information was systematically varied. Our general findings are as follows: (1) Prior to self-initiated "endogenous" saccades generated in the dark, there is a large, vertex-centered PSN which may be related to progressive activation of the SEF, (2) the PSN is greatly attenuated when the timing and direction of saccades are commanded by visual cues, (3) the SP always demonstrates a large negative focus over the lateral frontal electrode in the direction of the saccade suggesting that it is probably related to EMG activity from the underlying lateral rectus muscle (Thickbroom GW & Mastaglia FL, *Brain Res* 339:271-280, 1985), (4) this method provides dynamic information which complements that of cortical unit recording in subhuman primates and rCBF recording in humans using PET. These findings are generally consistent with the idea that the SEF may play a special role in self-initiated, endogenous saccades and provide support for the **dual premotor systems hypothesis** as applied to the generation of voluntary saccades.

496.16

TECTO-TEGMENTO-SPINAL CIRCUITS IMPLICATED IN SACCADIC HEAD TURNS IN THE BARN OWL (*TYTO ALBA*). T. Masino and E.I. Knudsen. Dept. Neurobiology, Stanford University, Stanford CA. 94305.

The barn owl makes quick orienting head turns towards auditory or visual objects of interest. The movement is thought to depend critically on the map of auditory and visual space in the optic tectum. To begin to understand how tectal activity controls head movements, we studied tecto-tegmento-spinal connections by injecting HRP or tritiated leucine into the tectum and HRP or rhodamine coupled latex beads into the spinal cord. Results indicate that, as in other avians, the direct tectospinal projection is extremely weak (avg. 3 cells per spinal injection). However, there are robust tectal projections to several regions of the brainstem tegmentum including the interstitial nucleus, the red nucleus, the gigantocellular reticular nucleus, regions around the oculomotor nuclei, and other sites within the midbrain and pontine reticular formation. Injections of retrograde tracers in the cervical spinal cord labeled cells throughout the brainstem and ventral thalamus. A comparison of anterograde label from tectal injections with retrograde label from the spinal cord injections revealed several regions of overlap, including the interstitial nucleus, parts of the red nucleus, and parts of the medial reticular formation.

We recorded electrophysiologically from cells in these brainstem regions while electrically stimulating the tectum. Single units were found which responded either with an abrupt increase or decrease of spontaneous firing rates or with a brief burst of spikes. Both short (6-10 msec) and long (12-30 msec) latency reponses were seen. Taken together, the anatomical and physiological observations suggest that tectal commands for saccadic head movement are mediated by several tegmental nuclei which operate in parallel to activate the spinal motor circuitry. (Supported by NIH grant R01-NS-16099.)

496.17

LOCALIZATION OF MOTONEURONS INNERVATING CERVICAL PREVERTEBRAL MUSCLES IN CAT AND RAT: IMPLICATIONS FOR CONTROL OF HEAD MOVEMENTS. L.J. Cruise and R.J. Cowie, Animal Section and Department of Anatomy, College of Medicine, Howard University, Washington, D.C., 20059.

A recent study of cat descending eye/head movement-related projections showed distinctive differences in caudal medullary and cervical spinal cord motoneuron (MN) targets (Holstege and Cowie, submitted *Exp. Brain Res*). While the location of MNs related to most dorsal and all superficial neck muscles could be identified from published accounts, an undefined medial target region seemed to contain MNs, possibly innervating deep prevertebral muscles (PVMs). Therefore, in this study PVMs including the longus capitis (LC), the rectus capitis ventralis and lateralis, as well as the epaxial obliquus capitis cranialis (OCC) were injected with HRP/WGA-HRP via a ventral surgical approach. Following 48 hr survival and standard aldehyde perfusion/postfixation, caudal medullary and cervical cord sections were reacted with TMB, mounted and counterstained, and the location and characteristic topography of the retrogradely-labeled MNs analyzed.

Preliminary results confirmed that PVMs in both species were typically innervated by MNs grouped along the ipsilateral medial gray/white margin (MMg) of the ventral horn, but not in the ventromedial nucleus. Labeled cell-columns in MMg partially overlapped dorsoventrally and extended caudally from the pyramidal decussation through the caudal end of C1. Occasional labeled cells were found in the C2 segment (or more caudally with LC injections), as well as within the anterior funiculus (AF), lamina X, and the opposite MMg. Labeled dendrites consistently extended medially into the AF, dorsally into laminae VII and X, and ventrolaterally toward lateral VIII and IX. Thus, the MNs projecting to the PVMs and OCC, which primarily act to flex, tilt and fixate the head, were found to be located optimally to intercept medial supraspinal pathways mediating control of head orientation, postural and compensatory reflexes.

(R.J.C. partially funded by a Faculty Research Support Grant, Howard University.)

496.18

NEURONAL ORGANIZATION FOR DIRECTED MOVEMENT IN THE FROG: SIMILARITIES IN VISUAL AND TACTILE PREY ORIENTING. P. Grobstein, K. Crowley* and J. Spiro*. Dept. Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

The movement triggered by a prey stimulus at a given location in visual space reflects not only the retinal region activated but other neural signals as well. We have found that a similar "activity gated divergence" characterizes the circuitry for tactile orienting. In different naturally occurring postures the rostral tip of the hindlimb lies at different locations relative to the body. In individual frogs, we repeatedly first recorded the location of this cutaneous site in a body coordinate frame, and then stimulated it by gentle stroking. For nearer and more rostral locations, the resulting movement was a directed snap. For more distant and caudal locations, frogs tended to respond instead with a reorienting movement lacking a snap component. We conclude that the orienting movement triggered by a given cutaneous stimulus is not invariant, but reflects instead an interaction of cutaneous signals with additional signals related to limb position in a body coordinate frame.

Retinal information is apparently transformed within the midbrain into a lateralized and parcellated signal representing stimulus location in a head or body centered coordinate frame. Unilateral tectal lesions produce scotomas in the contralateral visual field. More caudal unilateral lesions, in contrast, affect responses to stimuli in the ipsilateral hemifield, and yield a syndrome characterized not by scotomas but by a change in responses to those appropriate for more frontal locations. We have found evidence for similar organization in the case of tactile orienting. Following unilateral ventromedial lesions in the rostral medulla, frogs responded to ipsilateral tactile stimuli with turns of abnormally small amplitude; responses to contralateral stimuli were normal. Correlated with sufficiently reduced turn amplitudes was an increase in the frequency with which animals snapped, rather than reoriented, for more caudal stimuli. As with visual behavior, the lesions caused not abnormal movements but rather the normal movements appropriate for more rostral stimulus locations.

Our findings raise the possibility that the similarities between observations on tactile and visual behavior reflect shared use of common intermediate level circuitry carrying signals related to stimulus location in a non-sensory, non-motor coordinate frame. Consistent with this, we have found that when both visual and tactile behavior are studied in the same lesioned animals the observed visual and tactile deficits are correlated in occurrence and severity. Supported by NIH grant 1 R15 NS24968.

496.19

EFFECTS OF GUM HARDNESS ON VOLUNTARY CHEWING. B. Bishop, O. Plesh* and W. McCall. Depts. of Physiology and Oral Medicine, State Univ. of NY at Buffalo, Buffalo, NY 14214.

We reported previously that an individual's automatic chewing pattern is significantly modified with a change in peripheral feedback (i.e., a change in the hardness of chewing gum). In this study we asked whether the voluntary chewing pattern is similarly modified by changing gum hardness. We hypothesized that during voluntary chewing the central pattern generator which controls automatic chewing is by-passed, thus modifying the way peripheral feedback is centrally processed. Nine adults with no oro-facial dysfunctions were instructed to chew, in sequence, a standardized piece of soft and hard gum in time with a metronome set at 46, 100 or 160 BPM. We recorded jaw movements with a Kinesiograph and masseter EMGs with surface electrodes. Results showed that changing gum hardness evoked no changes in any temporal or spatial aspect of the chewing pattern or in the timing or recruitment of either masseter muscle at any chewing frequency. We conclude that neural mechanisms which process sensory feedback are by-passed when chewing is under voluntary control. (NIDR Grant DE-06717.)

496.20

LINEARITY & RELIABILITY OF JAW MUSCLE EMG & BITE FORCE. R.S. Kull, W.D. McCall, Jr., and D.C. Dixon*. Sch. Dent. Med.,SUNY, Buffalo, NY 14214.

The reports of linearity and reliability of jaw muscle EMG amplitudes with bite force are conflicting and scanty. We investigated this linearity and its reliability over two sessions.

Surface EMG activity was recorded from the anterior temporalis and masseter muscles on both sides in ten subjects while each bit on a transducer at the second molar using randomly ordered fractions of maximal isometric vertical force at two sessions separated by a week or more.

Scatter plots of EMG vs. force showed no consistent evidence of nonlinearity. Moreover, all 80 plots had $r>0.6$ ($p<.01$) with $r>.8$ in 80%.

Reliability was assessed visually by superimposing plots of EMG vs force for the two sessions and statistically by comparing the slopes of the regression lines at the two sessions. The plots superimposed well and the slopes did not differ significantly.

We conclude that masseter and temporalis EMG amplitudes are linearly related to bite force and are reliable over recording sessions.

Supported in part by USPHS Grant DE-07089.

496.21

SINGLE MUSCLE UNIT ACTIVITY DURING RHYTHMICAL JAW MOVEMENT. A. Lev-Tov, M. Tal and R. Lavy. Depatment. of Anatomy, The Hebrew Univ. Medical School, Jerusalem, Israel.

The present work characterizes the activity patterns of single muscle units during spontaneous rhythmical jaw movements (RJM) in ketamine anesthetized guinea pigs. Single unit recordings were obtained from the dorsal and ventral histochemical compartments of the anterior digastric muscle using teflon coated stainless steel wires (uncoated diameter= 90 μm).

The analysis of 5 different units (in 6 preparations) revealed 3 different patterns of activity: 1. continuous tonic activity at slow and regular firing rate (mean<25 Hz). 2. repetitive bursts of various durations at a slow and irregular firing rates. 3. high frequency (mean>63 Hz) rhythmical activity synchronized with the EMG bursts during RJM. The first two types of activity were observed mainly in the ventral compartment which is characterized by the abundance of slow-twitch muscle units (Lev-Tov, A. and Tal, M., *J. Neurophysiol.* 58:496, 1987) . The third type was evident in both compartments during the rhythmical EMG bursts and is likely to involve recruitment of fast-twitch motor units.

Further studies using refined recording techniques are now under progress.

496.22

COMPARISON OF SINGLE UNIT DISCHARGE PROPERTIES IN THE PERIAQUEDUCTAL GRAY AND NUCLEUS RETROAMBIGUUS DURING VOCALIZATION IN MONKEYS. E.A. DeRosier*, R.A.West*, C.R. Larson. Communication Sciences and Dirsorders, Northwestern University, Evanston, IL 60208.

Previous studies have shown that the midbrain periaqueductal (PAG) is involved in vocalization, but its precise function remains obscure, in part because of a lack of understanding of the location of vocalization-related PAG neuronal projections. Holstege (1987) demonstrated that PAG neurons project to the nucleus retroambiguus (NRA), and NRA cells project to the ventral horn of the spinal cord and the nucleus ambiguus, suggesting that the NRA may be involved in vocalization.

To test the hypothesis that the NRA may be involved in vocalization, extracellular recordings of NRA neurons were made in Macaca nemestrina monkeys trained to vocalize. Simultaneous recordings were made of the laryngeal and respiratory muscle activity and vocalization. Activity of the NRA neurons was evaluated with respect to vocalization and muscle activity and compared with PAG neuronal activity recorded under similar conditions.

In and around the NRA, cells with a variety of discharge patterns were observed, and some of these patterns were similar to those observed in the PAG. A few NRA cells were inactive and became active just before and during vocalization. Many cells displayed a respiratory rhythm and discharged either in phase with or out of phase with one or more of the respiratory muscles. Other neurons were phasically active with oral-facial movements and may be related to lingual, masticatory, facial, palatal or pharyngeal muscle activity. Cross-correlograms between units and EMGs indicated that NRA cells were more highly correlated with vocalization muscles than PAG cells. Microstimulation at PAG recording sites excited muscles with latencies of 12 - 20 ms. Microstimulation in the NRA excited muscles at 5 - 10 ms latencies.

The data support the idea that cells in and near the NRA are involved in vocalization. The NRA may serve as a relay and integration site for descending inputs to laryngeal and respiratory motoneurons.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CEREBELLUM III

497.1

CELLULAR PROPERTIES OF PURKINJE CELLS AND EXCITATORY TRANSMISSION IN THE ISOLATED TURTLE CEREBELLUM. N.T. Slater, D.R. McCrimmon and L.J. Larson-Prior. Dept. of Physiol., Northwestern University Medical School, Chicago, IL 60611 U.S.A.

The intact turtle cerebellum can be maintained *in vitro* for several days, allowing long-lasting (1-10 hr) somatic and dendritic intracellular impalements of Purkinje cells (PCs). We have studied the electrical properties of Purkinje cells in this preparation and the responses of Purkinje cells to activation of climbing- (CF) and parallel (PF) fibers. Somatic recordings of PCs revealed fast TTX-sensitive action potentials, slow calcium spikes, and a prominent 4-AP-sensitive outward rectification resembling A-current. Large plateau potentials were observed in solutions containing either TTX and $CdCl_2$, or TTX, TEA and 4-AP. Sodium spikes were largely absent in presumed dendritic recordings, but spontaneous bursting and calcium spikes were observed. All-or-none CF complex spikes could be activated by stimulation of the cerebellar peduncle, and PF-mediated EPSPs could be activated by 'on-beam' stimulation of the cerebellar margin. A novel slow EPSP was also observed following peduncle stimulation. The NMDA receptor antagonist DL-AP5 had little effect on these synaptic potentials, but the PF response was reversibly blocked by the broad spectrum excitatory amino acid (EAA) antagonist kynurenic acid (1-2 mM), thus EAA receptors may mediate transmission at the PF synapse. In summary, the data indicate that Purkinje cells in turtle cerebellum share many common electrophysiologic and pharmacologic properties with mammalian Purkinje cells *in vitro*. The resistance to anoxia of turtle brain will provide the opportunity to develop an intact brainstem-cerebellum preparation for routine intracellular recording. Supported by NS17489 and NS07243.

497.2

REGIONAL GLUCOSE UTILIZATION IS DIFFERENT IN THE BRAINSTEM AND CEREBELLAR NUCLEI OF GENETICALLY DYSTONIC AND NON-DYSTONIC RATS. J.F. Lorden and L.L. Brown. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294, and Dept. of Neurology, Albert Einstein Coll. of Med., Bronx, NY 10461.

A survey of brain glucose utilization (GU) was carried out to identify brain regions showing abnormal metabolism in the genetically dystonic (dt) rat, a mutant with a movement disorder involving twisting of the limbs and axial musculature. For 19 rats 19-24 days old, GU was estimated with arterial plasma data (Sokoloff, 1977). Animals were quiet during the experimental period. All 6 dt rats and 5 of 8 unaffected littermates had an overall GU rate lower than that of 5 normal non-littermates in the 61 regions analyzed (Mean difference, -37%). When the dt group was compared to the group of 5 unaffected littermates with similar low GU rates, the mutants had a significantly higher GU rate than controls in the deep cerebellar nuclei (e.g., n. interpositus, p .01). When a z-score analysis was used, differences were found in the deep cerebellar nuclei, locus coeruleus, pontine gray, red nucleus, principal nucleus of the third nerve, ventrolateral and ventromedial nuclei of the thalamus, lateral habenula and basolateral nucleus of the amygdala. The data suggest that the cerebellum and its efferents are the focus of abnormal neural activity in this mutant. (Supported by NS18062).

497.3

GLUTAMIC ACID DECARBOXYLASE (GAD) ACTIVITY AT CEREBELLAR PROJECTION SITES IN THE DYSTONIC RAT. G.A. Oltmans, M. Beales* and J.F. Lorden. Dept. of Pharmacology, Chicago Med. School, Chicago, IL 60064 and Dept. of Psychology, Univ. of Alabama, Birmingham, AL 35294.

Recent work has revealed the presence of a GABAergic projection from the deep cerebellar nuclei (DCN) to the inferior olive (IO) (Nelson, et al., 1984, Soc. Neurosci. Abst., 10:539), and lesions of this pathway produce a decrease in GAD immunoreactivity in the IO (Nelson & Mugnaini, 1985, Ibid., 11:182). In the genetically dystonic rat (dt) there is significantly increased GAD activity in the DCN, suggesting increased Purkinje cell inhibition of the intrinsic DCN neural activity. In the current study we measured GAD activity at two DCN projection sites, the IO and the red nucleus, to determine if this potential increase in inhibition of DCN activity would produce neurochemical consequences at the target sites. GAD activity in the red nucleus and IO was determined in discrete samples removed from frozen sections. In the IO a significant decrease in GAD activity was found in dt rats compared to normal littermate controls (-27%, p<.05), while no significant changes in GAD activity in the red nucleus were found. These results suggest that the elevated GAD activity found in the deep nuclei of dt rats may alter the activity of a GABAergic cerebello-olivary projection in this mutant. (Supported by NS18062 and the Dystonia Medical Research Foundation).

497.4

QUANTITATION OF CEREBELLAR CELL NUMBER IN REELER MUTANT MICE. Heckroth, J.A., D. Goldowitz, and L.M. Eisenman. Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA 19107. The reeler mutant mouse suffers from a serious genetically imposed defect in nervous system histogenesis, believed to be based on a generalized disruption of neuronal migratory mechanisms. The Purkinje cells of the reeler cerebellum are located in deep cellular aggregates, instead of in their normal superficial monolayer. Their abnormal position, and possible mixing with other cell types has precluded any study of their number in this mutant. We have used antiserum to the specific Purkinje cell marker, cGMP dependent protein kinase (gift of P. Greengard), to identify the Purkinje cells in reeler, and thus to quantify their number. Our results in four reeler mice indicate that this mutant possesses an average of 82,000 Purkinje cells, compared to 178,000 found in normal mice. Reeler Purkinje cell clusters are separate from the cerebellar nuclei of this mutant allowing us to quantitate the number of nuclear neurons as well. Our results indicate that reeler mice have an average of 8,500 nuclear neurons, a 15% reduction from the normal number of 10,000 cells. It thus appears that the nuclear neurons are not effected as severely as the Purkinje cells by the reeler gene, as their position is essentially normal, and their numbers are only slightly reduced. This finding is in line with studies of other mutations which seriously effect Purkinje cell number where little loss of cerebellar nuclear cells is observed.

497.5

c-fos EXPRESSION AND 14C 2-DEOXYGLUCOSE UPTAKE IN THE CEREBELLAR COPULA PYRAMIDIS DURING HINDLIMB MOTOR / SENSORY CORTEX STIMULATION IN THE RAT. Frank R. Sharp, Manuel F. Gonzalez, Stephen M. Sagar, James W. Sharp, Tom Curran*, and Kathleen R. Zahs. Departments of Neurology and Physiology, University of California at San Francisco and VA Medical Center, SF, CA 94121.

Electrical stimulation of hindlimb motor / sensory cortex of awake rats increased 14C 2-deoxyglucose (2DG) uptake diffusely as well as in granule cell patches in the dorsal and ventral lamellae of the eighth cerebellar hemispheric lobule, copula pyramidis (CP). Forelimb cortex stimulation and trained forelimb movements activated different regions of the paramedian lobule and copula pyramidis (CP). c-fos gene expression was examined using Fos protein immunocytochemistry 3 hours following a fifteen minute period of hindlimb cortex stimulation. Induction of Fos occurred in similar granule cell patches as those seen with 2DG. Fos was also induced in discrete Purkinje cell patches. Activated Purkinje and granule cell patches either wholly overlapped, partially overlapped, or were non-overlapping at various points along the CP lobule. The results support prior suggestions of compartmentalized processing of mossy fiber inputs and Purkinje cell outputs, and show that granule and Purkinje cell patches may be non-congruent as well as congruent.

497.6

MODEL OF LATERAL LINE SYSTEM FUNCTIONING IN THREE DIMENSIONS. Richard S. Babb, Rockefeller University, York Ave., N.Y., N.Y., 10021.

Previously, a simple theory of lateral line system functioning in the yaw plane has been proposed (Babb, 1982) based on work of Braitenberg (1967). Lateral line canals, the receptors of which are sensitive to low frequency vibrations, are found in the head as well as along the trunk of sharks. The canals of the head are found one above the orbit and another below. They are innervated by branches of the anterior lateral line nerve which projects to the lateral line lobe of the cerebellum. Phase information about waves detected by receptors in vertically separated ipsilateral canals is fed to the lateral line lobe and processed by the crista cerebellaris. According to the negative feedback model processing by parallel fibers in the crista would modify excitation in the plexuses inervating the lateral fin musculature. Movement of the fin musculature would produce a rotation of the shark's body in the pitch plane in a direction which would reduce the error signal. Such corrective action in the pitch plane along with that in the yaw plane would have the result of directing the shark in three dimensions towards the source of vibration.

497.7

DIFFERENT INFORMATION IS ENCODED BY SETS OF CLIMBING FIBER RECEPTIVE FIELDS IN THE ANTERIOR LOBE VERSUS THE PARAMEDIAN LOBULE. L.T. Robertson and G. McCollum. Robert Dow Neurological Sciences Institute, Portland, OR 97209-1595

The composition of climbing fiber receptive fields is related to the type of information encoded and to the ensembles of cells that participate in the encoding. An analysis was made of receptive fields of the face and the distal paw from individual climbing fiber responses encountered in the anterior lobe (AL) and paramedian lobule (PML) of the anesthetized cat. The boundaries of the receptive fields of the face or distal paw form compartments. Receptive fields of responses recorded in both regions are unions of compartments. Analysis of the inclusions of various compartments for the paw reveals that the lateral parts of the forepaw and hindpaw are included in a large proportion of the receptive fields for cells encountered in the PML, whereas the medial parts of the paw are more frequently included in forepaw receptive fields of cells located in the AL. Although many of the receptive fields are identified in both cortical regions, the proportion of the various compartments represented in the two regions are different. Thus, mechanical stimulation of certain parts of the paw or face will synchronously elicit different ensembles of climbing fiber responses in the AL versus the PML.

497.8

RESPONSE OF RAT PARAFLOCCULAR NEURONS TO COMBINATIONS OF VISUAL AND AUDITORY STIMULI. Brian N. Maddux, S.A. Azizi, and D.J. Woodward. *Dept of Cell Biology and Anatomy, UT Southwestern Medical Center, Dallas, Texas 75235.*

As part of an ongoing investigation into sensorimotor integration in the cerebellum, previous reports from this laboratory have demonstrated visual and auditory input to the rat paraflocculus. We have shown that these projections arrive via the dorsolateral basilar pontine grey from the peripheral regions of visual and auditory cortices, respectively, and that mossy fiber responses can be evoked to physiological stimuli in the unanesthetized preparation. In this study, recordings from parafloccular neurons were obtained with glass micropipettes in immobilized, locally anesthetized Long-Evans rats during visual and auditory stimulation. Selected images were projected onto a screen in front of the rat, while orientation, position, and velocity of travel were controlled by a computer. Delivery of a tone was also controlled by the computer, such that it was possible to deliver a variety of visual and auditory stimuli, each given alone or in combination with the other modality. Temporal offsets between paired stimuli can be varied systematically. The general finding is that combined auditory and visual stimuli may facilitate response of parafloccular neurons when compared to the response to single modality stimulus. Subthreshold inputs are clearly not "weak" inputs, since they are capable of powerful control over the dynamic range of firing when properly biased by input from a paired modality. *Supported by the Biological Humanics Foundation*

497.9

TRIGEMINAL NERVE SECTION IN NEONATES LEAVES HOLES IN CEREBELLAR GRANULAR LAYER TACTILE MAPS OF ADULT RATS. **M.G. Paulin and J.M. Bower, Division of Biology 216-76, Caltech, Pasadena CA 91125.**

The granular layer of crus IIa of the rat cerebellar cortex contains a fractured somatotopic map of perioral tactile receptive fields (lips, vibrissae, teeth etc). Because non-neighbouring sections of the body surface project to neighbouring regions (patches) in the cerebellar cortical map, we are interested in how the map is established ontogenetically. Previous work in this laboratory (Schlottman and Bower, Neurosci. Abstr. 13:77, 1987) showed that partial sectioning of the trigeminal nerve in 9-day postnatal rats causes crus IIa regions which contain patches responsive to tactile stimulation of the upper lip and vibrissae in normal rats to become weakly responsive to tactile stimulation of the upper lip near the nose and the corner of the mouth. Responses to these regions are not seen in crus IIa of normal rats, suggesting that partial nerve regeneration may have occurred. Accordingly, we have now used surgical procedures designed to ensure total nV section and to limit regeneration. In anaesthetized rats ranging from 2 to 11 days postnatal, we ligated the trigeminal nerve unilaterally and sectioned it distal to the ligation. At age 2-3 months, we mapped multi-unit responses in granular layer of crus IIa to tactile stimulation of the perioral regions, using glass micropipettes (1-2MW NaCl). The majority of rats had no responses, or very weak, nonspecific responses, in the regions normally occupied by upper lip projections. In a few rats we again found corner-of-mouth and perinasal responses as previously reported. As in previous experiments, the non-upper lip regions of the map appeared normal in all respects. These results further support the view that upper lip regions of the map are specified or reserved for the upper lip during development, and that, in general, the overall patchlike organization of tactile maps in cerebellar cortex is established inflexibly during development. (Supported by NIH grant 22205 and the Lucille P. Markey Foundation).

497.10

CEREBELLAR UNIT ACTIVITY DURING POSTURE AND MOVEMENT OF THE WRIST AGAINST CONSTANT TORQUE. Ted Milner & Allan M. Smith. Univ. of Montreal, Quebec, H3C 3J7.

A monkey was trained to move a wrist manipulandum to 3 target zones and then to maintain the position. We recorded wrist position, acceleration, torque, flexor EMG, extensor EMG and discharge of single cerebellar units while the monkey was: 1) moving against a constant flexor or extensor torque; 2) holding a target position against a constant flexor or extensor torque.

Conditions 1 and 2 elicited a reciprocal pattern of muscle activation from the prime movers of the wrist. When the torque opposed movement, activity in the agonist muscle gradually increased as movement progressed from the initial to the final position. When the torque assisted movement, activity in the antagonist muscle gradually decreased throughout the movement.

We have recorded from approximately 90 task-related units (including 15 Purkinje cells) in wrist and hand regions of the cerebellar cortex. Although the discharge rate for many cells was higher during movement than holding, inhibition did occur in some cases. Discharge rates were also related to EMG, target position, torque direction and movement direction.

497.11

LINEAR SYSTEMS ANALYSIS OF CEREBELLAR DEEP NUCLEI CELLS DURING PERFORMANCE OF CLASSICALLY CONDITIONED EYEBLINK. N.E. Berthier, A.G. Barto, and J.W. Moore. Depts. of Psychology and Computer and Information Science, Univ. of Mass., Amherst, MA. 01003.

Single unit activity was recorded from the interpositus and dentate nuclei of the cerebellum during classical conditioning the nictitating membrane (NM) response in rabbits. Lesions of interpositus and dentate disrupt both the performance and retention of CRs. We found that cells of anterior interpositus and medial dentate fire in relationship to CRs. This relationship was mathmatically modeled as a time-invariant linear system where cell firing frequency was the input to the system and nictitating membrane position was the output. For each cell we estimated the coefficients of a nonrecursive (transversal) filter that approximated the system using the criterion of least mean square error. We then used the filter to generate predicted NM position from the spike data. A Pearson correlation coefficient was then computed between the predicted position and the actual position of the NM. For those cells (N= 33) with r's greater than .5 transfer functions were computed. Nine of the transfer functions could be fit by first order equations with time constants ranging from 200 to 700 ms. The firing of these 9 cells was therefore predictive of both the position and velocity of the nictitating membrane. Fifteen transfer functions could be fit with second order equations with the damping ratio ranging from .4 to .9 and the undamped natural frequency ranging from 2.6 to 5.38 Hz. One transfer function was fit by a cascade of a first and second order system, and 7 were fit by fourth order equations. These results show that some cells of interpositus and dentate fire in a way that is very predictive of subsequent NM movement.

Acknowledgement: This work was supported by grants AFOSR 86-0182 AFOSR 87-0030 and NSF BNS 85-06989.

497.12

SYNAPTOGENESIS IN CEREBELLAR CORTEX OF ADULT RATS AFTER LESS THAN 15 HOURS OF VISUOMOTOR TRAINING OVER 30 DAYS. B.J. Anderson, K.R. Isaacs, J.E. Black, L. Vinci*, A.A. Alcantara & W.T.Greenough Depts Psych & Cell&Struct Biol, Neural&Behav Biol Prog, Univ Illinois, Champaign, 61820.

This work extends our previous report that visuomotor training increases cerebellar cortex thickness (Black, et al, 1987, Soc Neurosci, 13, 1596). We put 38 adult rats in 4 groups: an acrobatic condition (AC) given extensive motor-learning with relatively little exercise, two exercise conditions (FX, VX) that allowed little learning but given 10x the amount of AC exercise, and an isolated control group (IC). After 30 days blocks from the paramedian lobule were prepared for light and electron microscopy. The density of Purkinje cells under the molecular layer and synapse density in the molecular layer were then estimated. AC rats had significantly lower Purkinje cell densities, indicating substantial expansion of the molecular layer. Synapse density was nonsignficantly higher in AC rats, suggesting either that they had larger synapses or had more synapses per unit volume. Repetitive physical activity without learning (FX and VX groups) apparently has no effect on molecular layer synapses. In contrast, the expansion of molecular layer without apparent dilution of synapses in the AC group clearly indicates that visuomotor learning induces synaptogenesis in cerebellar cortex. Supported by NIMH.

497.13

THE EFFECT OF BILATERAL LESIONS OF THE CEREBELLAR NUCLEI ON MOTOR PERFORMANCE IN THE MONKEY. K. Amrani*, J.-P. Pellerin* and Y. Lamarre. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Canada H3C 3J7

Unilateral lesion of the dentate and interpositus nuclei produces severe movement dysfunction of the ipsilateral limbs which recovers in 10 to 15 days. The purpose of the present study was to determine if the intact contralateral cerebellar structures could be involved in this recovery process. Two monkeys (macaca sylvana) were trained to pick up morsels of food from the top of cylinders of different height attached to a rotating platform. A chronic chamber allowed for microelectrode recordings and electrolytic lesioning of the cerebellar nuclei on both sides. A first lesion of the nuclei on the right side produced a severe deficit in the right arm but did not affect the performance of the left arm. After total recovery of the right arm's performance 15 days later, the left cerebellar nuclei were lesioned. This produced a deficit in the left arm and also in the right contralateral arm that had recovered from the previous lesion performed on the right side. These results suggest that a functional relationship may exist between the two cerebellar hemispheres which could play a role in the motor recovery observed after unilateral cerebellar lesion. Supported by the MRC of Canada.

497.14

VELOCITY PERCEPTION IN PATIENTS WITH CEREBELLAR LESIONS. R.B. Ivry*, H.C. Diener, and S.W. Keele* (SPON: M. Flanders). Cognitive Neuropsychology Laboratory, Good Samaritan Hospital, Portland, OR 97210.

In three experiments, we examined velocity perception in patients with either degenerative damage or focal lesions in the cerebellum. The subjects were required to judge whether a line moving at a test velocity was faster or slower than a line moving at a standard velocity. In the first experiment, the test velocities ranged from 0.54 to 1.20 degrees/second. The subjects with cerebellar lesions were impaired in making the velocity judgments in comparison to age-matched control subjects. No differences were found between the two groups on a control task in which the position of the test line was varied with respect to a standard position. The second and third experiments explored a wider range of velocities. In addition, eye movements were monitored to examine whether the perceptual deficit is due to poor motoric control of eye movements. Preliminary results indicate that the perceptual deficit is observed at the higher velocities and that these findings can not be attributed to faulty eye movements.

497.15

INDUCED SLIP OF AN OBJECT HELD IN A PRECISION GRIP PRODUCES COMPENSATORY RESPONSES IN CEREBELLAR CORTEX AND THE MUSCLES OF PREHENSION. Claude Dugas and Allan M. Smith Centre de Recherche en Sci. Neurol. Univ. de Montréal, Montréal, Québec, Canada.

Monkeys were trained to lift and hold an object at a fixed height. During the static grasping a solenoid produced a downward slip of the object. With repeated trials two types of responses were observed; anticipatory responses & compensatory responses. Anticipatory responses consisted of an increase in the grip safety margin (grip force/load force ratio) and an increase in the rate of grip force application. Compensatory changes consisted of an increase in the EMG activity of muscles most directly related to grasping at a latency between 60 to 100 ms and a subsequent increase in the grip force. From a population of neurons recorded in the paravermal hand area of the cerebellar cortex about 40% of the neurons showed compensatory increases in activity between 40-60 ms. Some cerebellar neurons showed both anticipatory and compensatory responses. The results suggest that the cerebellum plays an important role in adjusting the grip forces to prevent both anticipated and unexpected object slippage between the fingers. Supported by the Medical Research Council of Canada and le Fonds F.C.A.R. du Québec.

497.16

THEORETICAL PREDICTIONS OF SPATIAL ANISOTROPY OF ACEREBELLAR DYSMETRIA IN HEAD MOVEMENTS OF CATS. *A.J. Pellionisz* and *B.W. Peterson* (Dept. of Physiology and Biophysics New York University Medical Center, New York, 10016 and Dept. Physiology, Northwestern Univ. Med.School, Chicago, IL, 60611).

The motor coordination function of the cerebellum has been characterized by ablation experiments, resulting in *acerebellar dysmetria*, since times of *Flourens* (1842) and *Holmes* (1939). Nevertheless, a quantitative mapping of dysmetria, measuring e.g. the well-known directional anisotropy of the misperformance has not been put forward, although comparison of measurements with predictions would yield direct experimental tests of theories on cerebellar function. The problem, in part, is the structural complexity of skeleto-muscular (sensori)motor systems; where an overdetermined number of joints and muscles co-act to produce a movement in a manner that is difficult both to predict and to measure. Tensorial modeling of the cat head-neck system *(Pellionisz and Peterson,* 1988, in "Control of Head Movements", Oxford Univ. Press) has facilitated theoretical predictions of acerebellar dysmetria in two ways.

First, tensor network theory puts forward a concise explanation of the coordination-function of the massively parallel cerebellar neuronal networks: *a geometrical metric tensor transformation of covariant intentional command into contravariant execution vectors,* cf. *Pellionisz,* 1985; in "Cerebellar Functions" (ed. *Bloedel et al.).* Thus, absence of the cerebellum would minimally affect movements into Eigenvector-directions of the coordinate system intrinsic to neck-muscle activations, (since for Eigenvectors co- and contravariant expressions are identical) while it would maximally distort movements furthest from these cardinal axes. *Second,* tensorial modeling yields predictions of the coordinate axes, intrinsic to head movements, even with several distributed centers of rotations, where the intrinsic multidimensional coordinate system (and its Eigenvectors) *change* with head-movements (*Laczkó et al,* 1987, Neurosci. Abst. 13:372). A video will show the *rotating* set of Eigenvectors, which specify the movements that would be least affected during acerebellar dysmetria. Methods of predicting precisely the errors during attempted movements in non-Eigendirections will also be presented. These predictions can be tested experimentally, and would provide further tests of the validity of current tensorial models of CNS function in multi-dimensional intrinsic sensorimotor coordinate frames. *Supported by: NS-22999*

497.17

HISTORY OF STEREOTAXY IN NEUROSCIENCE I. THE ORIGINS. L.H. Marshall and H.W. Magoun. Neuroscience History Program, Brain Research Institute, University of California, Los Angeles, CA 90024.

After Hitzig and Fritsch in Berlin demonstrated conclusively in 1870 that stimulation of points on the surface of the mammalian brain produced contraction of muscles on the opposite side of the body, important localization experiments were conducted in England. Responses form the cerebral surface of subhuman primates were mapped with a good deal of precision. Obtaining results from below the surface was neither precise nor reproducible. Victor Horsley, London's preeminent neurosurgeon, combined a thriving practice with laboratory experiments. He and Robert H. Clarke, a physician with engineering talent, attempted a study of function of the cerebellar nuclei. An improved technique was essential to control placement of the electrodes. Clarke applied geometric principles to the problem and designed and supervised the construction of the first instrument, which Horsley demonstrated in Canada at the annual meeting of the British Medical Association in 1906. The description of the Horsley-Clarke appeared in Brain two years later, but the experimental results were never published. The only important immediate use was by Ernest Sachs, a young American surgeon training with Horsley, who had a second instrument built and published his studies on the function and morphology of the optic thalamus. He then took the technique to America.

497.18

HISTORY OF STEREOTAXY IN NEUROSCIENCE II. REVIVAL. H.W. Magoun and L.H. Marshall, Neuroscience History Program, University of California, Los Angeles, CA 90024.

The experimental use of the Horsley-Clarke stereotaxic instrument, introduced in England in 1908, lapsed for almost 20 years and then was revived through an unusual chain of events. Shortly before he died in 1926, Robert Clarke, the instrument's inventor, urged Ernest Sachs, neurosurgeon at Washington University, St.Louis and owner of the second instrument constructed, to continue the studies on the cerebellum that Horsley and Clarke had begun in England. Sachs was conducting such studies during the short time that Stephen Walter Ranson and Joseph C. Hinsey were in St.Louis and saw the instrument in use. In 1928 they returned to Northwestern University and a copy was made of the model published in Brain and the worldwide use of stereotaxy in probing the brain from external landmarks was assured. Control of movement at several levels of the central nervous system was the first such exploration of so extensive a field. Before Ranson died unexpectedly in 1942, the role of the hypothalamus and lower brain stem in visceral integration, emotion, and the regulation of feeding,, fighting, sex, and other vital behaviors had been elucidated. The instrument was adapted to many animals, including man, and the technique improved. However, 80 years after Clarke, the determination of internal cerebral loci by external landmarks has been made obsolete by the new imaging techniques.

EFFECTS OF CHRONIC DRUGS

498.1

CHRONIC PRENATAL HALOPERIDOL EXPOSURE: EFFECTS ON D1 AND D2 DOPAMINE RECEPTOR BINDING IN MESOLIMBIC AND STRIATAL BRAIN REGIONS. B. Gough*, F.M. Scalzo, R.R.Holson and S.F. Ali (SPON: E.J. Peck, Jr.). Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Prenatal haloperidol (HAL) exposure in rats is known to reduce striatal D2 receptor binding sites in postnatal day 30 offspring. The effects of such HAL exposure on striatal D1 binding sites, and D1 and D2 binding sites in other regions of the dopamine (DA) system are not well described. Pregnant CD rats were given daily injections of HAL (2.5 or 5 mg/kg sc) or vehicle over gestational days 6-20. D1 and D2 DA receptor binding was measured in striatum, accumbens, amygdala and frontal cortex of male and female offspring sacrificed on postnatal day 30. In HAL treated animals there was a 20% decrease in both D1 and D2 binding sites in striatum, with a similar reduction in D2 but not D1 sites in accumbens. There was no HAL effect on D1 or D2 binding in amygdala. The data indicate that prenatal HAL exposure results in alterations in DA receptor binding that are specific to brain region and receptor subtype. The results further suggest that the developing nigrostriatal DA system may be more at risk for prenatal pharmacological insult than is the mesolimbic DA system.

498.2

CHRONIC PRENATAL HALOPERIDOL EXPOSURE: EFFECTS ON PRESYNAPTIC DOPAMINE AUTORECEPTORS. F.M. Scalzo, G. Newport*, and B. Gough*. Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Chronic prenatal haloperidol (HAL) treatment has been shown to have pronounced behavioral, psychopharmacological and neurochemical effects. Presynaptic dopamine (DA) autoreceptor function was assesed in the DA terminal rich regions nucleus accumbens, caudate nucleus and olfactory tubercles of rats exposed prenatally to HAL by measuring 3,4-dihydroxyphenylalanine (DOPA) and DA accumulation by HPLC following administration of the DOPA decarboxylase inhibitor NSD-1015. Pregnant rats were injected with either vehicle, 2.5 or 5.0 mg/kg haloperidol daily on gestational days 6-20. The offspring were treated with NSD-1015, NSD-1015 and gammabutyrolactone (GBL) or NSD-1015, GBL and apomorphine (APO) prior to sacrifice on postnatal day 97. There appeared to be no impairment of autoreceptor function in the caudate of animals exposed prenatally to HAL as indicated by the ability of APO to attenuate GBL induced increases in DOPA accumulation. However, HAL treated animals exhibited increased DOPA accumulation following treatment with NSD-1015 and had smaller relative increases in DOPA accumulation following NSD-1015 and GBL treatment compared to vehicle injected controls. These data suggest that prenatal HAL exposure causes long-lasting alterations in the adult dopamine system.

498.3

EFFECT OF INJECTION SCHEDULE ON TOLERANCE TO HALOPERIDOL-INDUCED "ANOREXIA." D.L. Wolgin. Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33431

We previously found that rats given daily injections of haloperidol became tolerant to the initial "anorexic" effect whether the drug was given before or after access to the food. These results suggest that tolerance is not contingent on access to food in the drugged state. However, because the drug is long-acting, it is possible that the group given the drug after food access was actually intoxicated during testing. To control for this possibility, rats in the present study were given the drug either before or after access to milk on alternate days. Control rats were given injections of saline. On the intervening days, all groups were given milk in order to monitor baseline levels of intake. Contrary to previous findings, little tolerance was observed in either drug group on test days, although baseline intake was constant. These results suggest that injection schedule plays an important role in tolerance to haloperidol-induced "anorexia."

498.4

DECREASED STRIATAL(S) ACH RELEASE FOLLOWING CHRONIC HALOPERIDOL(H) TREATMENT **E. Friedman, H-Y Wang and P. Butkerait* Medical College of Pennsylvania, Philadelphia, PA 19129.**

The effect of chronic H treatment on K^+-evoked [^{3}H]ACH release from superfused S slices was assessed. While acute and chronic H produced increases in S [^{3}H]ACH release, H withdrawal following chronic treatment produced decreases (34-38%) in evoked [^{3}H]ACH release. SKF-38393 produced dose-dependent (0.1-10uM) increases in S [^{3}H]ACH release which were blocked by SCH 23390 and by bicucculine. The effect of D_1-receptor stimulation was significantly reduced after 2.5 and 5 mo of H treatment. Both LY171555 and carbachol produced dose-dependent (0.1-10uM) inhibitions of S [^{3}H]ACH release. Long-term treatment with H (2.5 and 5 months) elicited increased sensitivity to the effect of LY-171555 while the effect of carbachol was diminished only following a 5 mo treatment period. The apparent desensitization of presynaptic muscarinic receptors coincided with a 19% decrease in Bmax of S muscarinic receptors labelled by [^{3}H]N-methylQNB but not of those labelled by [^{3}H]pirenzepine. These findings demonstrate that withdrawal from chronic H produces a hypocholinergic state in the striatum. These findings suggest that diminished S ACH release observed following discontinuation of long term H treatment may contribute to the emergence of tardive dyskinesia.

498.5

HALOPERIDOL-INDUCED VACUOUS CHEWING MOVEMENTS IN THREE DIFFERENT RAT STRAINS J.M. Dale*, C.A. Tamminga, L. Goodman*, N. Kaneda*, H. Kaneda* (SPON:W.T. Carpenter). MPRC, University of Maryland, Balto., MD, 21228

Chronic treatment of rats for 6-12 months with neuroleptics has been proposed as an animal model of tardive dyskinesia. We have examined this paradigm by studying haloperidol (HAL)-induced vacuous chewing movements (VCM) in three different rat species. 38 male rats were treated, comprised of 3 strains: Sprague-Dawley (SD), Long Evans (LE), and Wistar (W). Nine rats of each strain were treated with 1.5 mg/kg/day of HAL orally through their drinking water; four (3W) were water treated. For behavioral measurements, animals were observed individually for four consecutive 30 sec periods and VCM's quantified. Rats were examined weekly beginning 6 weeks after initiation of HAL and continued for a total of 25-27 weeks. After 20 wks the animals were challenged with atropine 5 mg/kg IP and rated for VCM at 0, 30, 60, and 120 min. At wk 20-22 the rats were weaned from HAL and rated for 2 wks. HAL treated rats from all three strains had a significant increase in VCM/2min compared with controls (C): 8.3xC (SD); 4.7xC (LE); and 6.2xC (W). Atropine decreased VCM in all groups by 27%, but VCM remained significantly above control levels. VCM in all groups remained apparent after neuroleptic withdrawal, with only a 17% decrease in SD rats. Thus, VCM's in rats approximated the pharmacologic behavior of tardive dyskinesia, but there also appear to be differences between rat strains. Biochemical analysis of brain samples from these treatment groups may suggest reasons for rat strain differences.

498.6

REVERSIBILITY OF TARDIVE DYSKINESIA FOLLOWING NEUROLEPTIC WITHDRAWAL A.C. Lahti*, G.K. Thaker, J.A. Nguyen*, M.Moran*, C.A. Tamminga (SPON: R. Lahti). MPRC, University of Maryland, Balto., MD, 21228

Tardive dyskinesia (TD) is a hyperkinetic motor disorder which is thought to be secondary to neuronal changes following chronic receptor blockade by neuroleptics in the basal ganglia. Recently it has been suggested that the process is reversible if the dopamine (DA) blockade is removed. To further explore this issue we carried out a long term follow-up (mean = 10.5 months) of 20 TD patients (mean age = 43.7 ± 7.2 yrs.), who had been withdrawn from their neuroleptic treatment during the follow-up period.

The study demonstrated that there was a marked decrease in TD (average reduction of 43.5 ± 32.3%) in 17 patients during the neuroleptic free period. Three patients who did not show improvement were all elderly (>65 yrs) females. Multiple regression analysis revealed a significant negative correlation (partial $r = -0.38$, $p<0.03$) between the age of the patients and their observed change in TD score. There was also a significant correlation between the duration of NL free period and the change in TD scores (partial $r = 0.41$, $p<0.03$).

These results strongly suggest that TD is reversible, especially in young patients. Further, they emphasize the utility of atypical neuroleptics (e.g. clozapine), which purportedly exert low or no DA receptor blockade in the basal ganglia, in the treatment of psychotic patients with TD.

498.7

COMPARISON OF SHORT- AND LONG-TERM HALOPERIDOL ADMINISTRATION AND WITHDRAWAL ON PEPTIDE LEVELS IN THE RAT. J.M. Radke, A.J. MacLennan, M.C. Beinfeld*, G. Bissette, C.B. Nemeroff, S.R. Vincent, & H.C. Fibiger. Div. Neurological Sciences, U.B.C., Vancouver, B.C., Canada; Dept Pharmacology, St. Louis University, St. Louis, MO, USA; Dept. Psychiatry and Pharmacology, Duke University.Durham, NC, USA.

The effects of oral administration of haloperidol (1.3-1.5 mg/kg/day) for 3 weeks (short-term), 8 months (long-term), and 2 month withdrawal after 8 months (withdrawal) on the levels of somatostatin, substance P, cholecystokinin (CCK), and neurotensin were examined in the rat. Short-term administration produced changes in peptide levels similar to those reported by others, with the most pronounced changes being decreased somatostatin levels in the ventral tegmental area, the striatum and the nucleus accumbens, decreased substance P levels in the substantia nigra and nucleus accumbens, increased CCK levels in the ventral tegmental area and medial prefrontal cortex, and increased neurotensin levels in the striatum and nucleus accumbens. With a few exceptions, similar observations were found following long term-term haloperidol adminstration. In contrast, withdrawal after long-term administration resulted in most peptide levels returning to control values, with the exception of neurotensin which was significantly decreased in the striatum and nucleus accumbens.

498.8

DRUG DISCRIMINATION TRAINING DURING CHRONIC DRUG TREATMENT AFFECTS THE DEVELOPMENT OF TOLERANCE. K. L. Burgin*, W. F. Caul and R. J. Barrett (SPON: M. H. Ebert). Vanderbilt University, Nashville TN, 37240.

Psychoactive drugs produce distinct primary and compensatory adaptive processes that occur in temporal sequence. These adaptive processes are thought to be the basis for pharmacodynamic tolerance. The purpose of this experiment was to evaluate the extent to which continued drug discrimination training during chronic treatment effects the development of tolerance. Rats were trained to discriminate distilled water from 0.75 mg/kg amphetamine in a two-lever drug discrimination task. Two groups were then given a chronic drug regimen of 13 daily injections of either distilled water or 10 mg/kg amphetamine. Drug discrimination training was continued for half of each chronic drug group. Tolerance was observed only for the group that was not trained during the chronic amphetamine treatment. The data show that (a) choice responding after chronic drug treatment is not influenced by the amount of training per se, and (b) rather continued training during chronic treatment provides the opportunity for reinforced responding to shifting drug and nondrug cue states.

498.9

EFFECT OF ANTIDEPRESSANT DRUGS ON SUBTYPES OF CENTRAL BETA ADRENOCEPTORS: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. **G.A. Ordway*, C. Gambarana*, J. Bensler & A. Frazer, VA Med. Ctr. & Univ. of Pennsylvania School of Medicine, Philadelphia, PA, 19104.**

Repeated administration of many antidepressants to rats reduces the density of beta adrenergic receptors (BARs) in the cerebral cortex, as revealed using ligand binding techniques on homogenate preparations. Whether a similar effect is seen throughout the brain has not been well characterized. We have used in vitro quantitative autoradiography to measure subtypes of BARs (Proc. Natl. Acad. Sci. (USA) 81:1585, 1984) in brains of rats treated chronically with either the tricyclic antidepressant desipramine (DMI), the inhibitor of type A monoamine oxidase clorgyline, or the selective inhibitor of the uptake of serotonin, citalopram. Of 23 areas examined, treatment with DMI reduced the total binding of ^{125}I-iodopindolol (IPIN) significantly ($P<0.01$) in 20 regions and clorgyline had a significant effect in 16 regions. This reduction in the binding of IPIN was due almost exclusively to a decrease in IPIN binding to the beta-1 subtype. In DMI-treated rats, the beta-1 subtype was reduced significantly in 17 regions and in 12 areas of clorgyline-treated rats. DMI-treatment reduced beta-2 adrenoceptors in only one area and clorgyline treatment did this in three areas. In most areas, the quantitative effect of clorgyline on the beta-1 subtype was much less than that caused by DMI except in the paraventricular n. of the thalamus and the basolateral amygdaloid n.. Treatment with citalopram caused no significant effect on the binding of IPIN to either subtype of BAR in any area. **Supported by Research Funds from the Vet. Admin. and USPHS grant MH29094.**

498.10

BEHAVIORAL PROFILE DURING AMPHETAMINE WITHDRAWAL IN RATS: A MODEL OF DEPRESSION? D. B. Neill, G. K. Mumford*, P. R. Hartley, S. A. Murray* and G. W. Vogel*. Depts. of Psychology and Psychiatry, Emory University, Atlanta, GA 30322.

Amphetamine or cocaine withdrawal has often been reported to produce depression in humans. We investigated whether this happens in rats because of the possibility of subsequent neurobiological analysis. Because of the anhedonia in depressed humans, most of our tests focused on the rats' reactivity to rewarding stimuli. Baseline data were acquired for intracranial self-stimulation (ICSS), food-rewarded bar-pressing on fixed-ratio (FR) 20 and fixed-ratio 100 schedules in food-deprived rats, intake of a palatable food in nondeprived rats, locomotor activity, and male sexual behavior. Behavioral analyses were then stopped during 10 days of twice daily administration of 10 mg/kg *d*-amphetamine sulfate. Testing resumed during the first day of withdrawal. Locomotor activity and ICSS performance dropped 50% during withdrawal. Performance on FR was unaltered. Intake of the palatable food and sexual behavior were enhanced. We conclude that, while portions of this behavioral profile are consistent with a depression model, other portions are not. The data are most easily explained by the view that a given behavior is altered during withdrawal in a way opposite the way it is altered during acute amphetamine administration.

498.11

EFFECTS OF CHRONIC LITHIUM TREATMENT ON RECEPTOR-MEDIATED INOSITOL PHOSPHOLIPID HYDROLYSIS AND PROTEIN PHOSPHORYLATION IN RAT BRAIN. T. Casebolt* and R.S. Jope, (SPON: A.L. Beckman), Dept. of Pharmacology, Univ. of Alabama, Birmingham, AL 35294

The etiologies of mania and depression, and the mechanism of action of lithium as a treatment for these disorders, are unknown. To test the hypothesis that a part of the therapeutic effect of lithium is due to altered receptor-coupled phosphoinositide (PI) hydrolysis, rats were treated with LiCl (0.17% in diet, 30 days) and agonist-induced PI hydrolysis was measured in rat brain. Norepinephrine, but not carbachol-induced PI hydrolysis was significantly reduced after chronic lithium treatment. Experiments examining the mechanism of this effect led to the hypothesis that receptor number or receptor-coupling was reduced by chronic lithium treatment. Examination of the effects of chronic lithium treatment on α_1-adrenergic binding of [^{3}H]prazosin and chlorethylchlonidine indicated that α_1-adrenergic receptor subtypes were not altered by chronic lithium treatment.

The possible role of protein phosphorylation in receptor uncoupling after chronic lithium treatment was also examined. First, the distribution of PKC activity in membrane and cytosol fractions was measured. Endogenous protein phosphorylation mediated by three kinases was also surveyed after chronic lithium treatment. Supported by MH 38752.

498.12

OPIOID RECEPTOR UPREGULATION INCREASES THE REWARD VALUE OF LATERAL HYPOTHALAMIC BRAIN STIMULATION. *S. Uysal* and E. E. Coons** (SPON: L. Kiorpes). Department of Psychology, New York University, New York, NY 10003.

We have examined the effects of naltrexone-induced opioid receptor upregulation (two 30 mg pellets s.c. over 9 days) on lateral hypothalamic self-stimulation (SS), stimulation-induced feeding (SIF), and stimulation-induced escape (SIE). Opioid receptor supersensitivity following naltrexone withdrawal potentiated SS while SIF and SIE were unaffected. The observed increase in SS was reversed by acute injection of naltrexone, demonstrating opioid receptor mediation. Upregulation-induced potentiation of SS occurred only at electrode sites that did not support SIE. These were posterior to placements supporting SIE, as previously observed (S. Carden and E. E. Coons, 1987).

While receptor upregulation-induced supersensitivity to opiates has been shown previously (M. T. Bardo et al., 1983, B. C. Yoburn et al., 1985), this is the first report we are aware of that demonstrates supersensitivity to endogenous opioids. Furthermore, though past research has shown that opioids can modulate SS (K. D. Carr and E. J. Simon, 1984), the present results indicate that under conditions of supersensitivity opioids may mediate SS as well. Paradoxically, while SIF is mediated by opioids under baseline conditions (K. D. Carr and E. J. Simon, 1983), it is unaffected by upregulation.

498.13

INTRANIGRAL INJECTION OF NALOXONE PRECIPITATES WITHDRAWAL IN MORPHINE-DEPENDENT RATS. A.A. Baumeister, T.G. Anticich* and M.F. Hawkins. Department of Psychology, Louisiana State University, Baton Rouge, LA 70803.

Recent evidence (Baumeister et al., Brain Res., in press) suggests that the substantia nigra (SN) is an important site of action of systemically administered morphine. The present study sought to determine whether this nucleus is involved in the development of physical dependence to this drug. Male Sprague Dawley rats received subcutaneous (sc) injections of morphine sulfate suspended in a sustained release preparation (Collier, et al., Nature, 237, 1972, 220) according to the following schedule: 40 mg/kg (day 1); 60 mg/kg (day 2); 80 mg/kg (day 3), and 100 mg/kg (day 4). Twenty hours after the last sc injection animals received a bilateral intranigral injection of naloxone (1 - 10 µg/ 0.25 µl saline) or saline (0.25 µl). Intranigral injection of naloxone produced several significant ($p<.05$) signs of withdrawal in morphine-dependent animals including wet dog shakes, teeth chattering, squealing and diarrhea. No withdrawal signs were observed in morphine-dependent animals that received intranigral saline or in nondependent controls that received intranigral naloxone. There was a significant correlation ($p<.01$) between naloxone dose and magnitude of the response for each withdrawal symptom. These results suggest that the SN mediates the development of physical dependence produced by repeated administration of morphine. (Supported by USPHS grant HD-21560).

498.14

EFFECTS OF CHRONIC CAFFEINE ON RAT BRAIN MONOAMINES. T.R. Taylor*, D.G. Kirch*, G.A. Gerhardt, N. Benowitz*, C. Stephen*, R. Freedman and R.J. Wyatt. National Institute of Mental Health, Washington, DC 20032

Caffeine was administered in three doses (10 mg/kg/day, 25 mg/kg/day and 50 mg/kg/day) to rats as twice-daily I.P. injections for 30 days. Plasma caffeine concentrations and regional brain concentrations of monoamines [dopamine (DA), norepinephrine (NE) and serotonin (5HT)] and their metabolites [dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-hydroxyindoleacetic acid (5HIAA)] were determined. In striatum, data was obtained for all the above except MHPG. In frontal cortex and cerebellum, NE, 5HT and their metabolites were determined. In addition, for each monoamine an index of turnover was calculated [metabolite(s)/monoamine].

A linear dose-response relationship was found for plasma caffeine concentrations. At 10 mg/kg/day no monoamine changes were found except for a decrease in cerebellar NE. At the higher doses there were significant changes in all three brain areas. Striatal DOPAC decreased significantly, while DA increased (with a trend toward a lower DA turnover index). The 5HT index decreased significantly in striatum and cerebellum, with a similar trend in frontal cortex. The NE index decreased in frontal cortex, but increased in cerebellum (the only increase observed in the turnover indices).

498.15

EFFECT OF ORAL CAFFEINE INGESTION ON WHEELRUNNING IN RATS. **R.E. Landrum, T.A. Landrum, and C.J. Meliska. Department of Psychology, Southern Illinois University, Carbondale, IL 62901-6502.**

Twenty-four male Sprague-Dawley rats were housed continuously for 24 days in Wahmann activity wheels. Eight rats received continuous access to 0.5 mg/ml caffeine base in tap water; 8 received continuous access to tap water; and 8 received 24 hr access to 0.5 mg/ml caffeine alternating with 24 hr access to tap water. Mean wheel revolution data for the caffeine/water (C/W) group revealed a significant decrease in wheelrunning on water days compared to controls receiving continuous water, suggesting a withdrawal reaction to the absence of caffeine. When raw data were transformed into percentages of baseline wheelrunning, continuous access to caffeine failed to stimulate, and actually inhibited wheelrunning slightly, relative to water controls. For rats alternating between caffeine and water (C/W), wheelrunning was significantly greater on caffeine days than for all other conditions including continuous caffeine access. These data suggest that when given orally, caffeine's stimulatory effects on wheelrunning are particularly sensitive to the context and scheduling of drug administration.

498.16

CONTINGENT INEFFICACY AND TOLERANCE TO CARBAMAZEPINE IN AMYGDALA-KINDLED RATS. S.R.B. Weiss* and R.M. Post. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

The relationship of the timing of drug administration to anticonvulsant efficacy against amygdala-kindled seizures was studied. During kindling development, rats received either no treatment or carbamazepine (15 mg/kg) before (carba-before) or after each stimulation (carba-after). After kindling to full seizures, all animals received carbamazepine before the stimulation. Both the drug-naive and carba-after group showed a good anticonvulsant response. The rats that received carba-before during kindled seizure development remained unresponsive (conditioned inefficacy). The two groups of responsive animals developed tolerance to carbamazepine's anticonvulsant effects (contingent tolerance), which could be reversed by switching to carba-after or kindling the animals drug-free, but not by carbamazepine administration alone or time off from both drug and seizures. Another group of rats were kindled with carba-after or vehicle-after to determine whether prior exposure to carbamzepine in a noncontingent fashion would facilitate the subsequent development of tolerance. When these rats were switched to carba-before treatment, both groups developed tolerance to carbamzepine at equal rates.

These data suggest that tolerance to or inefficacy of anticonvulsant medications may be learned phenomena susceptible to modulation through alterations in the temporal relationship between drug administration and seizure induction.

498.17

IMPAIRMENTS RELATED TO LONG-TERM USE OF DRUGS IN CHRONIC PAIN PATIENTS. D.M. Kwilosz*, L.E. Tune, G.D. Pearlson, P.J. Walsh*, P.H. Dorsey*, & M. Nossel* Psychiatry Dept., Johns Hopkins University School of Medicine, Baltimore, MD 21205

Cognitive effects of long-term use of benzodiazepines and opiates in chronic pain patients are poorly understood. Less is known about the influence of short-term fluctuations in drug levels due to metabolism. Information about persistent effects of long-term use and fluctuations due to high vs. low levels may help to identify impediments to effective pain management. The purpose of the study is to determine neuropsychological and phenomenological correlates of naturally occuring variation in medication levels.

Inpatients on a chronic pain treatment unit were tested approximately 2 hrs post administration (peak) of benzodiazepines and/or opiates and just prior to administration (trough) weekly for three weeks. The test battery included measures of psychomotor ability, distractability, memory, pain, and mood. Drug levels were measured via a saliva sample using radioreceptor assay techniques.

Preliminary results show poorer performance at peak than trough levels in psychomotor speed, set shift and in learning-memory, although anxiety and pain levels did not vary significantly. The findings suggest that the short-term fluctuations in opiate/benzodiazepine levels may affect neuropsychological abilities contributing to functional incapacity in chronic pain patients.

498.18

ALTERED MATERNAL BEHAVIOR AFTER CESSATION OF CHRONIC MORPHINE ADMINISTRATION DURING PREGNANCY. E. LaCrosse and B. Culver. Neurosci. Prog., Dept. of Psych. & Sch. of Pharm., Univ. Wyoming, Laramie, WY 82071

Disruption of normal maternal behavior is an important variable in studies designed to assess functional alterations in offspring of animals administered morphine sulfate (MS) during pregnancy. A number of studies in the literature report acute MS-induced changes in maternal behavior. Our lab has shown behavioral alterations in offspring of rats administered MS during pregnancy. It is possible that cessation of chronic MS administration during pregnancy alters maternal behavior which subsequently influences the development of the offspring. We report here the results of a study designed to examine this possibility.

Rats were implanted with 1 MS-silastic pellet (100 mg) on gd 14, and 2 more were implanted on gd 17. Paired controls (LA) were implanted with silastic lactose pellets (100 mg) on the same days. Parturition was designated as day 0. The pellets were removed on day 1. Maternal behavior was observed on days 2 through 5. Results show that cessation of MS administration disrupts maternal behavior on a number of indices.

These results indicate that there are alterations in maternal behavior associated with cessation of MS administration. The presence of wet-dog shakes suggests that MS withdrawal contributes to these alterations. Previous results from our lab show high neonatal mortality and persistent behavioral alterations in offspring of rats administered MS during pregnancy. Findings in the present study, suggest that alterations in mother-pup interactions associated with cessation of chronic MS administration may contribute to the increased mortality and alterations in the behavioral development of the offspring.

498.19

SUBCHRONIC EFFECTS OF +MDMA ON NEUROTRANSMITTER RELEASE IN THE RAT. R.E. Maloney*, B.K. Yamamoto, and M.D. Schechter (Spon.: E. Krimmer) Dept. of Pharmacology, Northeastern Ohio Univers. Col. of Med., Rootstown, OH 44272.

MDMA (5-20 mg/kg) has been shown to be neurotoxic to the serotonergic system with a minimal effect on the dopaminergic system. Subchronic treatment with low doses however, increased both serotonin and dopamine (DA) in specific rat brain nuclei (Maloney et. al., Neurosci. Abst., 1987). To further investigate these findings, rats were administered either 1.5 mg/kg +MDMA (**D**) or 1.0 ml/kg H_2O (**V**) i.p. for 6 weeks according to the following 2 week injection schedule: DVVDDVDDVV. Between 2 and 3 weeks after the last injection, DA release was measured from anterolateral caudate by in vivo microdialysis in these awake, behaving rats. Dialysis perfusion (2.5 µl/min) was with a modified a modified Ringer's solution (pH 7.25). Samples were collected every 20 min and analyzed for DA, DOPAC, and HVA by HPLC/EC. After a 1 hr stable baseline, all rats were injected with 1.5 mg/kg +MDMA. Dialysate samples were collected for 3 hrs. There was a significant increase in DA accompanied by a decrease in DOPAC. Forty min after MDMA, DA release in the subchronically treated MDMA group was significantly higher (204% of baseline + 19) than the vehicle-treated group (146% + 16) ($p<0.05$). There were no differences in DOPAC levels between these groups at this time. These results may indicate a sensitization to the DA releasing properties of MDMA following an intermittent, low dose treatment regimen. Future studies will assess the effects of this treatment regimen on neurotransmitter content in other brain nuclei. Supported by grant DA04181-01, M. D. Schechter, P.I..

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY VI

499.1

A MATHEMATICAL MODEL FOR EMMETROPIZATION IN THE CHICKEN. H. C. Howland and F. Schaeffel*. Section of Neurobiology and Behavior, Cornell University, Ithaca N.Y. 14853.

Eye growth and refractive state in the chicken has been examined under a large number of experimental treatments. Degrading the retinal image by use of occluders produces myopia, although with a high variability in individual refractions. "Image degradation myopia" can be also produced in birds without accommodation (due to lesions in the Edinger Westphal nucleus), with the optic nerve sectioned and, by local image degradation, in local parts of the visual field. Recovery occurs in both normal and EW-lesioned birds. In addition, we have shown that eye growth compensates for an imposed refractive error induced by spectacle lenses in both normal and EW lesioned chicks. Most of the results listed above argue for regulation of eye growth by local mechanisms within the eye, without the neccessity of accommodation. However, we have developed a computer simulation for regulation of eye growth in the chick which showed that there are probably (at least) two independent feedback loops controlling eye growth, one within the eye and the other dependent on accommodation.

499.2

THE VITREOUS HUMOR IN EXPERIMENTALLY INDUCED MYOPIA R.L.Seltner* J.J. Pastemak* J.G.Sivak* (SPON. R. Beauchamp) School of Optometry and Department of Biology, University of Waterloo, Waterloo, Ont. CANADA N2L 3G1

Experimentally induced myopia can be produced rapidly in chicks subjected to post-hatching blur of the retinal image. This myopia is characterized by axial and equatorial eye size increase, increase in wet weight of the eye, and a large negative refractive error, within 14 days of post-hatching goggle application. Previous results have indicated that the changes in size occur as a result of expansion of the vitreous chamber.

We have studied the volumetric changes in the vitreous humor in chicks subjected to vision blur for a 14 day period, and the reversibility of these changes after removal of the goggle. As well, the proteins of the vitreous humor have been examined for qualitative and quantitative changes accompanying the development of myopia.

In chicks, the vitreous humor is made up of 2 parts, a gel and a liquid portion. In the myopic eye, the gel:liquid ratio of the vitreous humor, as well as the total liquid vitreous increases dramatically when compared to the contralateral control eye. These effects are reversible after removal of the goggle.

Results using PAGE and protein assay methods indicate that the protein concentration in the myopic eye is decreased when compared to the non-myopic eye. There appears to be no change in the banding pattern of the proteins in the myopic eye, although the intensity of the bands is decreased when compared to the non-myopic eye. The effects observed suggest that the vitreous humor is playing a role in experimental myopia, and may also play a role in the normal post-hatching emmetropization mechanism.

This research is supported by the Natural Sciences and Engineering Research Council of Canada.

499.3

ASSESSMENT OF SPATIAL VISION AND VISUAL FIELDS IN NATURALLY STRABISMIC MONKEYS. M. W. Quick,* M. Joosse* and R. G. Boothe (SPON: J. Tigges). Yerkes Regional Primate Research Center, and Departments of Psychology and Ophthalmology, Emory University, Atlanta, GA 30322.

A screening program has yielded monkeys having a naturally occurring ocular misalignment. Its occurrence was often associated with a large hyperopic refractive error, and with a difference in refractive error between eyes. Quantitative estimate of the deviation was made from photographs of the corneal reflex. Results showed monkeys with 1) alternating fixation; 2) deviations that varied with fixation angle; 3) deviations that varied with fixation distance, indicating an accommodative component.

Spatial vision was assessed operantly. Grating acuity results showed a sensory deficit in the deviating eye of some monkeys, with differences up to one octave when compared to the fixating eye. Further deficits were seen in tests of optotype acuity. This further reduction correlated with deficits in phase discrimination and increased spatial distortion. Also, a loss of sensitivity to contrast was evident at mid and high spatial frequencies. The horizontal extent of the visual fields were determined using a perimetry apparatus. Deficits were noted within the monocular segment of some non-preferred eyes, indicating field losses in deviating eyes which are unaccounted for by typical competitive mechanisms. Correlations will be drawn between oculomotor state and behavioral results. NIH grants EY-06436 and RR-00165.

499.4

BEHAVIORAL CONTRAST SENSITIVITY OF PRENATALLY UNILATERAL ENUCLEATED CATS. S. Bisti*, M.C. Cenni*, L. Maffei*, C. Trimarchi* (SPON: European Brain and Behavior Society). Ist. di Neurofisiologia del CNR, 56100 Pisa, Italy.

Prenatal unilateral enucleation in the cats leads to a complete reorganization of the visual pathways. At the level of visual cortical area 17 all neurones are driven by the remaining eye. Visual receptive fields have normal selectivity for orientation and directionality, but are smaller on average than those of normal cats. In a previous study we showed that the visual acuity of denucleated cats, measured both behaviorally and by VEPs, is similar to normal cats (Perception,16:332, 1987).

Here we report contrast sensitivity as a function of spatial frequency for two prenatally monocular enucleated kittens, measured both behaviorally and by VEPs. The results obtained by both methods show that whereas the visual acuity is comparable to the monocular acuity of normal cats, maximum contrast sensitivity is somewhat higher, and the peak in the contrast sensitivity function occurs at higher spatial frequencies.

499.5

VERNIER ACUITY, GRATING ACUITY AND CONTRAST SENSITIVITY IN EXPERIMENTALLY STRABISMIC MONKEYS. D.C. Kiper, L. Kiorpes and J.A. Movshon. Department of Psychology and Center for Neural Science, New York University, New York, 10003.

Human strabismic amblyopes show an abnormal relationship between vernier acuity and grating acuity. We studied this relationship in strabismic monkeys to better understand the neural basis of amblyopia. In addition, we investigated the hypothesis that vernier acuity can be predicted from knowledge of the contrast sensitivity function.

We measured monocular vernier acuity, grating acuity and contrast sensitivity for 7 monkeys (Macaca nemestrina) made esotropic at ages ranging from 3 to 8 weeks. All data were collected using operant methods. The relationship between vernier acuity and grating acuity in the strabismic monkeys was different from that of normal adult monkeys. However, the relationship in strabismic monkeys could be described by the performance of young normal animals. The deficits in vernier acuity were not obviously associated with the contrast sensitivity functions.

499.6

INFANT PERCEPTION OF ROTATION FROM RIGID STRUCTURE-FROM-MOTION DISPLAYS. R.V. Spitz*, J. Stiles-Davis* and R.M. Siegel (SPON: S. LeVay). Dept. of Psychology, University of California, San Diego, La Jolla, CA 92093 and The Rockefeller University, New York, NY 10021.

The term structure-from-motion refers to a class of phenomena in which perception of a structure can be abstracted from the integration of unconnected moving elements. In recent work it has been demonstrated that both adult humans and monkeys are capable of perceiving a rigid 2- or 3-dimensional structure from such displays (Siegel & Andersen, 1988). The present study seeks to extend these results through a longitudinal examination of infant ability to abstract a 2-dimensional rigid structure from displays similar to those used in the adult primate studies.

A habituation procedure was used involving two displays: one structured display and one unstructured display. The structured display consisted of 128 points which appeared to lie on the surface of a disc rotating about an axis perpendicular to its surface. The unstructured display was created by taking these same points and shuffling the set of motion trajectories from the structured display, thus the unstructured display appeared as random-motion. The displays were identical except for the presence or absence of the rotating structure, and this structure could only be perceived by integrating a set of relative motion cues across the array. Eighteen infants were tested longitudinally at 4-, 5-, 6-, and 7-months of age. Each infant viewed one of the displays for 23 8-sec trials and was then tested for dishabituation with 5 trials for the other display. The results of this study indicate that it was not until 6-months of age that infants demonstrated any dishabituation to the novel display indicating that they could discriminate the structured from the unstructured displays. This is in spite of the fact that infants as young as 4-months of age are capable of using local relative motion cues to specify an edge (Spelke, 1985). Thus, it appears that the integration of a set of spatio-temporal cues may be a later appearing phenomenon, developmentally, than is the perception of the cue itself. Future work should further extend this by examining infant ability to integrate other spatio-temporal cues.

499.7

POSITRON EMISSION TOMOGRAPHIC (PET) STUDY OF EFFECT OF AMBLYOPIA AND OPTICAL BLUR ON HUMAN CORTICAL RESPONSE TO COMPLEX VISUAL STIMULATION. J. L. Demer, N.D. Volkow*, G. K. von Noorden, K. L. Gould*. Cullen Eye Institute, Texas Children's Hospital, and Positron Diagnostic and Research Center, Houston, TX, 77030.

Amblyopic animals are known to have a paucity of neurons in primary visual cortex that can be visually stimulated via the affected eye. We used PET (resolution 12-14 mm full width at half maximum) to investigate effects of visual stimulation on relative glucose metabolism in cerebral cortex of normal and amblyopic human subjects using the tracer [^{18}F]deoxyglucose. The visual stimulus was a monocularly-presented dramatic motion picture. Two control subjects had normal vision in each eye, while 3 amblyopes had monocularly decreased vision ($\leq$20/200) and normal vision in the fellow eye. Relative glucose metabolism was quantified by dividing activity in regions of interest by total activity in the entire brain image slice.

Repeated scanning demonstrated similar relative glucose metabolism in primary visual cortex of a control viewing with either normal eye. Optical blur (~20/200) reduced relative glucose metabolism in primary visual cortex by ~8% in two controls. In two amblyopes, relative glucose metabolism in primary visual cortex was 5-6% less during stimulation of the amblyopic than the sound eye. In one amblyope, a combination of amblyopia and optical blur reduced relative glucose metabolism in primary visual cortex by 23% relative to the sound eye. Four of 5 subjects exhibited asymmetrically greater relative glucose metabolism in the contralateral hemisphere (particularly the temporal lobe) during visual stimulation of sound or amblyopic eyes; optical blur reduced the degree of asymmetry. The remaining subject exhibited persistently greater relative glucose metabolism in the right hemisphere.

These preliminary results indicate that **amblyopia reduces visual activation of relative glucose metabolism in primary visual cortex** of amblyopic humans; this effect may be **simulated by optical blur** in normal subjects. In both normal and amblyopic subjects, complex visual stimulation often produces **asymmetrically greater relative glucose metabolism in the contralateral hemisphere;** this effect is **reduced by optical blur.**

Supported by the National Children's Eye Care Foundation, the Delta Gamma Foundation, the Clayton Foundation for Research, and USPHS grant EY-06594.

499.8

DEVELOPMENT OF LUMINANCE MODULATED VISUALLY EVOKED POTENTIALS TESTED WITH PSEUDORANDOM BINARY SEQUENCE STIMULI. **R. Srebro and M. Hutchison-Clutter*. Dept. of Ophthalmology, UT Southwestern Med. Cntr., Dallas, TX 75235-9057.**

Visually evoked potentials (VEP) were obtained from 45 healthy normal full-term infants from 4 weeks to one year of age, 6 normal and 6 amblyopic adults. The stimulus was pseudorandom binary sequences (PRBS); .8 to 66.7 Hz at 100% modulation depth, identical to band-limited white noise except for having a periodicity of 1.0s allowing signal averaging. The stimulus was presented by means of a yellow LED array subtending an homogeneous 10^{0} field at a mean luminance of 6 foot-lamberts. Each test session consisted of 32 monocular presentations (approximately 41s total). A Laplacian electrode derivation centered at Oz was used. Fourier amplitude spectra and input-output cross correlation functions (CCF) were calculated from the averaged VEP. The highest driving frequency (F_D) of the VEP was estimated by calculating CCFs using band-pass filtered (2-pole Butterworth) copies of the stimulus with 3 dB cut-offs ranging from 1 to 40 Hz. Both the Fourier amplitude spectra frequency maxima and F_D shifted towards higher frequencies with increasing age. However, adult levels were not reached by the end of the first year of life. These results may reflect the development of central nervous system mechanisms, whereas previous studies using flicker fusion may reflect retinal cone development. Differences between normal and amblyopic adults were found.

499.9

VISUAL CORTEX MAPS IN KITTENS WITH BILATERAL AMBLYOPIA. Max S. Cynader[1], Joanne A. Matsubara[1], Nicholas V. Swindale[1]*, Kathryn M. Murphy[2]*, and Donald E. Mitchell[2]. Dept. of Ophthalmology[1], U.B.C., Vancouver, B.C. V5Z 3N9, and Dept. of Psychology[2], Dalhousie University, Halifax, N.S. B3H 4J1, Canada.

We studied visual cortex functional topography in kittens reared under a deprivation regime which results in reduced visual acuity. Kittens were reared with one eyelid sutured until five weeks of age. Thereafter, they had 9 or 18 days of reverse suture, during which time the initially-deprived eye was allowed vision, while the initially exposed eye was occluded. Thereafter, both eyes were allowed normal vision. This results in a profound deterioration of vision in both eyes, with visual acuity reduced by several octaves. We searched for anomalies in visual cortex functional maps in these animals. Using multiple, closely spaced electrode penetrations, we studied cortical magnification factor, receptive field size, local receptive field scatter (the point-spread function), and the layout of ocular dominance, orientation, and direction selectivity across the cortical surface. We found that the gross properties of the cortical map are normal, despite the amblyopia. Most importantly, there appears to be no difference in the retinotopic order of the map (point-spread function).

A striking difference was found in receptive field size. In the amblyopes, receptive fields averaged 60% larger than those of normal cats. This highly significant difference, coupled with the equality of point-spread function, leads to large differences in the cortical area over which receptive fields overlap and thus in hypercolumn dimensions between normal and amblyopic animals.

499.10

INTRACORTICAL AND GENICULOCORTICAL CONNECTIONS IN CATS WITH SEVERE BILATERAL AMBLYOPIA. J.A. Matsubara, D.E. Mitchell & M.S. Cynader. Depts. of Anat. & Psych., Dalhousie University, Halifax, N.S., B3H 4H7, Canada.

The local visual cortical and geniculocortical connections in cats with severe bilateral amblyopia were studied. Kittens were reared under conditions similar to those previously described, but with variations in the frequency and duration of daily reverse occlusion, resulting in an even greater deterioration in visual acuity. Microinjections of WGA-HRP or Con A were pressure injected into areas 17 or 18. Coronal sections of the LGN and tangential sections of the cortex were processed using the TMB method or the aviden-biotin method following incubation in a primary antibody directed against Con A.

The maximum number of patches surrounding an injection in area 18 was reduced from 10, in normal animals, to 4 in bilateral amblyopes. The extent of the labeling was also reduced along the M-L but not the A-P cortical axis. Thus, patches were distributed in a long, thin strip running A-P, a pattern reminiscent of one seen in bilateral enucleates. Finally, while the intracortical connections were reduced, preliminary results on the geniculocortical connections seemed abnormally widespread, with labeled cells occuring in over 60% of the LGN after a microinjection in area 18. These results suggest that the larger size of receptive fields recorded in bilateral amblyopes may result from the abnormally widespread geniculocortical projection. This work supported by the M.R.C. (Canada).

499.11

DEVELOPMENT OF FLASH EVOKED RESPONSES IN NEONATAL RAT SUPERIOR COLLICULUS. S.K. Itaya and S. Molotchnikoff. Dept. Biomed. Sci., Univ. So. Ala., Mobile, AL 36688, and Dépt. des Sci. Biol., Univ. de Montréal, Montréal, Qué.

We looked for the earliest flash evoked responses in the superior colliculus of neonatal rats. Anesthetized hooded rat pups had the cortex exposed and the opposite eye opened. Glass microelectrodes (1-2 Mohms) were used to measure field potentials. The stimulus was a strobe flash about 0.5 m above the head. Responses were recorded in the usual manner, averaged over 32 to 64 presentations. Two ERGs were measured, and two optic nerves were stimulated directly. Some brains were examined microscopically for the electrode track. No responses were observed before postnatal day 12 (P12). On P12, in 2 of 4 rat pups, flash evoked responses were recorded in superior colliculus with a biphasic potential of very long latency (250-350 msec) compared to the adult (about 50 msec). Responses were recorded within 500 um of the collicular surface. ERGs were first seen on P12, as has been reported. On P13, all 4 pups showed stronger collicular responses and also responses in visual cortex. At P14, when eyes are normally still closed, there were collicular and cortical responses with increased amplitudes and decreased latencies, e.g., 180-200 msec in the colliculus. We did not observe reversal of potential polarity. Action potentials in response to the light were first observed in the colliculus on P14.

499.12

DEVELOPMENT OF FLASH EVOKED RESPONSES IN THE ECTOSTRIATUM OF THE ZEBRA FINCH:
J. Engelage* and H.-J. Bischof* (SPON: EBBS).
University of Bielefeld, Dept. Ethology, POB 8640, D-4800 Bielefeld 1, F.R.G.

Recently a sensitive phase for the development of the tectofugal system has been demonstrated in our laboratory by anatomical methods. We are able to demonstrate the development of VEPs in the telencephalic target area of this pathway. In 20d old birds we recorded simple negative waves with low amplitudes. The typical pattern of the CSD depth profiles of adults could not be observed in these birds. VEPs recorded in the ectostriatum of 40d old birds showed the negative-positive wave typical of sexually mature birds with amplitudes within the adult range. However, there were differences in the shape of the VEP curves in 40d old birds and adults. CSD profiles showed a typical source-sink-source pattern comparable to that in adults. In the ectostriatum of 40d old birds reliable ipsilateral VEPs could be recorded similar to those of adult birds. Although anatomical data suggest that the development and the first sensitive phase are complete by d40, we conclude that physiological changes occur up to d60. Supported by the DFG.

TROPHIC AGENTS VII

500.1

A MUSCLE-DERIVED FACTOR PROMOTES SURVIVAL OF EMBRYONIC MOTOR NEURONS IN CULTURE. S.J. Jeong, T.H. Oh and G.J. Markelonis. Dept. of Anatomy, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Survival of embryonic spinal motor neurons requires a muscle-derived trophic factor(s). We have partially purified a non-laminin protein from muscle tissues which stimulates neurite outgrowth (Oh, T.H. et al., Dev. Biol., in press); we are now investigating whether this protein promotes survival and growth of motor neurons. Cultures enriched in motor neurons were prepared from chick embryos using metrizamide density gradients. Using retrograde labeling techniques, we confirmed that the large cells recovered were motor neurons. Most of the large, putative motor neurons were found in metrizamide fraction I. We then determined the quantitative influence of the protein on survival and neurite outgrowth of cultures enriched in motor neurons over longer periods. When motor neurons in fraction I were plated on polylysine-coated dishes, the protein was able to support the survival of a proportion of the motor neurons plated. These large cells also extended neurites in culture in the presence of the protein. Laminin alone supported survival of a small proportion of motor neurons plated, but the level of survival was much lower than that by the protein. Both the protein and laminin promoted survival and neurite outgrowth of cells in fraction II suggesting that the survival effect of the protein was not specific for motor neurons. (Supported by NIH grants NS15013 and NS20490).

500.2

SENSORY AND SPINAL CORD NEURONS CULTURED IN SERUM-CONTAINING OR DEFINED MEDIA: IMMUNOHISTOCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES. D. S. Grega and T. J. Cavanagh*. Boehringer Mannheim Corp. Biochem. R&D, Dept. Cell Biol. Indianapolis, IN 46250

Serum-free or defined medium (DM) provides a more reproducible environment than serum-containing medium (SCM) for studying neural regeneration in culture. We have devised several DM for primary neural culture and here report immunohistochemical (IHC) and electrophysiogical data. Spinal cord and dorsal root ganglia (DRG) cultures were prepared from 12-14 rat embryos and maintained at least 3 weeks. SCM was compared with several DM's (proprietary formulations). IHC studies consisted of staining neurons (NSE), astrocytes (GFAP) and oligodendrocytes (GalC). The morphology and relative frequency of cell types in the various media were noted. Electrophysiological recordings were made from DRG neurons. Resting potential, action potential amplitude, duration, and afterhyperpolarization were compared. At 4 weeks in culture, there were no significant differences between DM1 and SCM cultures. Further comparisons of differing DM formulations are planned.

500.3

POSSIBLE NEUROTROPHIC EFFECTS OF NIMODIPINE ON COGNITIVE BEHAVIOR AND HISTOCHEMICAL DISTRIBUTION OF AChE and B-50 (GAP43) IN UNILATERAL FIMBRIA-FORNIX LESIONED RATS. A.M. Danks*, A.B. Oestreicher*, R.L. Isaacson, B.M. Spruyt*, W.H. Gispen*. Institute of Molecular Biology and Medical Biotechnology, Univ. of Utrecht, Utrecht, NL.

Male Wistar rats (220-240 g body weight) were subjected to left unilateral fimbria-fornix transection or sham operation. For seven days beginning the day of surgery, the rats received i.p. injections of 0.06, 0.5, 1.0, or 5.0 mg/kg nimodipine (Bayer AG, Leverkusen, FRG), or vehicle.

Open field and water maze behavioral measures were taken on days 11, 12, and 13 for animals sacrificed 14 days post-surgery; or days 25, 26, and 27 for animals sacrificed 28 days after surgery. Lesioned animals showed a significant learning impairment in the water maze, as compared to sham-operated controls, and the differences were not attributed to motor deficits since the animals did not differ in open field tests of locomotion. Spontaneous recovery of function was revealed by a reduced acquisition impairment in the water maze in rats allowed to recover 28 days.

Histochemical analysis revealed a reduction in AChE staining in medial septum, hippocampus, and n. accumbens brain regions. To examine differences in the expression and distribution of B-50 (GAP43), immunohistochemistry was performed. Effects of the lesion and nimodipine on behavior and histology will be discussed.

500.4

GM1 RESTORES DOPAMINE AND ACETYLCHOLINE IN THE BRAIN OF DEVELOPING RATS AFTER HYPOXIA. T.A. Wemlinger*, L.A. Isaacs*, H.S. Weiss, N.H. Neff, A.J. Yates and M. Hadjiconstantinou, Depts. of Pharmacology, Physiology and Pathology, The Ohio State Univ. Col. of Med., Columbus, OH 43210.

Acute or chronic hypoxia alters neurotransmitter function in the brain. Perinatal hypoxia causes neonatal brain damage resulting in various symptomatologies. Neurotrophic factors are a new generation of therapeutic agents for treating degenerative diseases of brain. There is now considerable evidence suggesting that administrating exogenous GM1 ganglioside promotes the recovery of neuronal function after various lesions. We now report that hypoxia causes long-lasting neurochemical changes in various regions of the brain of developing rats and that administration of GM1 corrects these deficits. Five day old rats were exposed to hypoxia (8% oxygen remained nitrogen) for 2 h and the animals killed 24 h or 3 weeks later. Twenty-four h after the insult dopamine (DA) and its metabolites were reduced in the striatum, frontal cortex and hippocampus. Three weeks later DA content recovered in the striatum and hippocampus but was still reduced in the frontal cortex. DA uptake studies followed the same pattern. Similarly, hypoxia caused a long-lasting reduction of acetylcholine (ACh) content and choline uptake in the striatum and frontal cortex of developing rats. Pretreatment with GM1 partially prevented the short term neurotransmitter changes after hypoxia. Furthermore, continuation of the drug for 3 weeks resulted in full restoration of DA and ACh content as well as DA and choline uptake.

500.5

MYOBLAST PROLIFERATION INDUCED BY SERUM FROM PATIENTS WITH HYPERTROPHIC MUSCLE. K.E. Misulis, C.M. Stoscheck*, & K.A. Jaeckle*. Neuromuscular Disease Res. Ctr., Vanderbilt Univ., Nashville, TN 37212

We have studied sera from patients with idiopathic muscle hypertrophy for the presence of factors which may influence myoblast proliferation and differentiation.

Mouse primary myoblast cultures were prepared from 1 day-old mice. Heat-inactivated sera from patients were applied to the myoblast cultures and incubated for 5 days. Subsequently, the myoblasts were removed by trypsin and EDTA and counted in a hemocytometer. The results from the experimental sera were compared to those using sera from normal volunteers.

Myoblasts incubated in sera from normal subjects had an increase in cell number over the 5 days to 574% of plated number. Patient serum resulted in an increase in myoblast proliferation to 1330% of the pre-incubation cell number. Serum levels of many hormones, including growth hormone, thyroxin, somatomedin C, were normal.

These data suggest that myoblast proliferation may be controlled by an as yet unidentified circulating factor, and this may be present in excess in patients with idiopathic muscle hypertrophy.

Supported by NIH-CIDA grant #NS01134 to Dr. Misulis and a VA research grant to Dr. Stoscheck.

500.6

ACTH 4-10 ANALOGUE ENHANCES RECOVERY FROM BRAIN INJURY Brain Res. Lab., Clark U., Worcester, MA 01610 & Biomeasure, Inc., Hopkinton,MA 01748. M.J.Attella*, S.W.Hoffman*,J.E.Taylor&D.G.Stein

Male Sprague-Dawley rats 90-100 days old were given lesions of frontal cortex and administered s.c. either sterile water (SW), 1µg, 10µg, or 100 µg of ACTH 4-10 analogue (BIM-22015) every other day for twenty days. Seven days later they began testing in the Morris water maze to locate a submerged platform. Brain-injured subjects given either 10µg treatment or SW took significantly more trials than sham operates to reach the platform eight consecutive times, while rats with lesions given 1µg or 100µg performed better than lesion controls. No significant differences were found in distance or time taken to reach the platform. After eight days, the subjects' ability to recall the location of the platform was determined. No differences were found in trials to reach the platform eight consecutive times. Percent savings formula performed on mean time to locate the platform revealed that sham operates did not differ from subjects with lesions given 1µg, 10µg, or 100 µg of the analogue. However,lesion controls exhibited negative percent savings. Thirty days after the last injection (42 days post-injury), the rats were trained in a delayed-spatial alternation task to assess long-term effects of the treatment. Brain-injured rats initially treated with SW or 1µg took more days to score 9 out of 10 correct alternations than sham or lesion subjects given 10µg of the analogue.With respect to the mean errors to this criterion, animals given 10µg again showed superior performance over those with lesions given SW or 1µg. Rats with comparable brain injury treated with 100µg made fewer errors than the 1µg group but did not perform better than lesion controls. A count of Acetylcholinesterase-labeled neurons in the nucleus basalis of Meynert (NBM) showed that brain-injured rats treated with either SW, 1µg or 100µg of the analogue exhibited significantly fewer neurons in comparison to sham operates. However, brain-injured rats given the 10µg treatment did not differ from the sham controls with significantly more neurons in the NBM than brain-injured counterparts treated with SW or 100µg.

500.7

NEUROMODULATION OF CATECHOLAMINERGIC GROWTH IN THE CHICK EMBRYONIC BRAIN BY TWO HYPOTHALAMIC NEUROPEPTIDES. S. Kentroti and A. Vernadakis (SPON: D.G. Whitlock). Depts. of Pharmacology and Psychiatry, Univ. of Colorado Sch. of Medicine, Denver, CO 80262

We are investigating the neuromodulatory role of growth hormone-releasing hormone (GHRH) and gastrin-releasing peptide (GRP) on catecholaminergic neuronal expression in the chick embryonic brain. The activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis, was used as an index of neuronal development. The peptides were given either at embryonic days E1 to E7, a period of neuronal differentiation, or E12 to E14, a period of synaptogenesis. In the first paradigm, animals received GHRH (135ng) or saline vehicle (50ul) in ovo on days E1,3,5 and 7 and sacrificed on E8. Whole brains minus optic lobes were assayed for TH activity, expressed as pmoles $^{14}CO_2$ liberated/h/mg protein. TH activity was significantly higher ($p<.001$) in the treated embryos versus controls (4.60±0.46 vs 1.95±0.13, respectively). A second group of animals received GRP (375ng) or saline vehicle in ovo on E12 and E14 and animals were sacrificed on E15. TH activity was significantly higher ($p<.05$) in cerebral hemispheres from treated versus control embryos (7.06±1.72 vs 4.83±0.58, respectively). We interpret these data to mean that GHRH and GRP may be involved in catecholaminergic neuronal growth and synaptic function. (Support:NIH grant #AA07464)

500.8

TREATMENT OF GANGLIOSIDES AND AMPHETAMINE FAIL TO REDUCE SPATIAL LEARNING DEFICITS IN A WATER-MAZE TASK FOLLOWING BILATERAL LESIONS OF THE CAUDATE NUCLEUS. G. L. Dunbar#, L. Lescaudron*, B. S. Bitran*, S. A. Hecht*, and D. G. Stein. Brain Research Lab, Clark University, Worcester, MA 01610. #Dept. of Psychology, Central Michigan University, Mt. Pleasant, MI 48859.

Although gangliosides (GM1 and AGF2) and d-amphetamine reduce learning deficits on a two-choice, spatial reversal task after bilateral caudate nucleus (CN) lesions (Dunbar et al., Soc. Neurosci. Abstr., 12:1283, 1986), the effects do not generalize to a task which requires utilizing distal cues (i.e., the Morris water maze). Forty-one male, albino rats (Sprague Dawley, 350-450g) were given sham operations and IP saline injections (n=9) or bilateral CN lesions, followed immediately by IP injections of either 30 mg/kg GM1 (n=9), 20 mg/kg AGF2 (n=7), 2 mg/kg d-amphetamine (n=7) or physiological saline (n=9). Injections were given daily for 10 days. Five days after surgery, testing began in the Morris water maze and in an open field twice a day, for five days. None of the treatments were able to reduce lesion-induced deficits on the water maze task. Also, no between-group differences were found in open-field activity levels. These results suggest that the beneficial effects of gangliosides and amphetamines may be task-specific in reducing spatial learning deficits following CN lesions.

500.9

5,7-DHT-LESION-INDUCED NEUROTROPHIC FACTORS INCREASE SURVIVAL OF GRAFTED 5-HT NEURONS IN THE CEREBELLUM. F.C. Zhou and E.C. Azmitia Dept. Anatomy, Indiana Univ., Indianapolis, IN 46223

5,7-DHT (a 5-HT neurotoxin)-lesion in an afferent to hippocampus induces sprouting of 5-HT fibers in the hippocampus from another afferent (Azmitia et al., Nature, 274:374, 1978; Zhou and Azmitia, Brain Res., 308:53, 1984; 373:337, 1986). This 5,7-DHT-altered hippocampus was found favorable to the growth of grafted 5-HT neurons but not norepinephrine neurons. Furthermore, extracts from the altered hippocampus promotes the growth of grafted 5-HT neurons (Zhou et al., J. Neurosci. Res.17: 235, 1987). In the present study, the trophic extracts were found to support the survival of the grafted 5-HT neurons in the cerebellum where fewer 5-HT fibers innervate and grafted 5-HT neurons seldom survive.

Dissociated fetal raphe cells (1.1×10^6) from E-14 were grafted into the cerebellum (right side) of adult albino rats with neurotrophic extracts, NTF3 (1:1 or 1:100) as experimentals or with Hank's solution as control; all animals were examined 6 weeks after surgery. Both immunostaining of 5-HT neurons (0, 0, 0, 0, 51, 54 stained neurons, n=6) and HPLC measurement of 5-HT level (right/left =0.11/0.10 ng/mg, n=4) confirmed that grafted 5-HT neurons with Hank's vehicle do not survive well in the cerebellum. Grafts supplemented with NTF3, 1:100 dilution, have 86, 246, and 307 (n=3) stained 5-HT neurons. Grafts supplemented with NTF3, 1:1 , have no survivals (n=3). Furthermore, the grafted 5-HT neurons with NTF3, 1:100, have noticeably higher density of 5-HT fibers than those in control.

Current results and previous observations suggest that high-dilution NTF3 promotes growth of 5-HT fibers in favorable, (hippocampus) as well as in unfavorable (cerebellum) environments. NTF3 further supports the survival of grafted 5-HT neurons when they were placed in the unfavorable cerebellum. Finally, highly concentrated NTF3 may contain a factor inhibiting neuronal growth. Supported by NIH RO1- 23027-02.

500.10

TROPHIC SUPPORT OF ALBUMINS ON CENTRAL NEURONAL CELLS IN VITRO. D. D. SVRZIC* (SPON: J. PATRICK),Dept. of Biol. M-007, University of California, San Deigo, CA 92093.

Pure cultures of central neurons in which all cells stained positive for neurofilaments were obtained from dissociated whole chick forebrain on embryonic day 8. The cultures were grown for 1, 5 and 7 days in vitro either on untreated tissue culture plastic or on polyornithinlaminin precoated plates. We observed trophic support of bovine serum albumin (BSA) and ovalbumin (OA) when present in the serum free medium in the concentration range from 15 nM to 1.5 mM and from 0.87μM to 28 μM. respectively. At the plateau level of BSA (75μM) 66% of the cells survived for more than 24 h of incubation, whereas at the plateau level of OA (13.5 μM) only 52% of the cells survived. This neuronotrophic support exceeded by far our observation of the effects of pyruvate/catalase in the presence of a buffer in the short term cultures. Only the albumins provided an extended neuronotrophic support (7 days) which was accompanied by the appropriate neuronal sprouting. Neuronal survival was estimated by means of a strip-counting technique and an automated colorimetric micro-assay (Manthorpe, M., et al., Develop. Brain. Res. 25: 191, 1986).

Albumins of a greater purity have excluded the possibility of essential fatty acids being responsible for the described effects. It remains to be determined if the forms of somatomedins: IGF-I and IGF-II (Carlsson-Skwirut, C., et al., FEBS 201:46, 1986) are the trophic substances that permit not only the longevity of the neurons, but also the normal maintenance in culture.

500.11

NEURITE-PROMOTING ACTIVITY FROM THE BRAIN OF MPTP-TREATED MONKEYS WITH AND WITHOUT SURGICAL LESIONS. Margaret A. Palmatier, Robert Plunkett*, Kryztof Bankiewicz, Alexander C. Cummins*, and Irwin J. Kopin. CNB, NINCDS, NIH, Bethesda, MD. 20892

Cerebrospinal fluid (CSF) or gelfoam pellets from caudate cavities of untreated or unilaterally MPTP-treated Macaque monkeys were tested for neurite-promoting activity. Neurite-promoting activity was assayed using chick dorsal root ganglion (DRG) from 8 to 9-day-old chicken embryos explanted onto laminin-coated tissue culture wells in serum-free medium in the presence of various amounts of CSF. Ganglia were scored after 18 hours for neurite outgrowth relative to neurite outgrowth induced by various concentrations of Nerve Growth Factor (NGF) in the same bioassay. CSF from untreated monkeys did not induce neurite outgrowth from chick DRG. CSF from MPTP-treated monkeys induced neurite outgrowth, as did CSF and gelfoam pellets from monkeys that had received a surgically placed cavity in the caudate nucleus. These results extend the finding of lesion-induced, brain-derived neurite-promoting activity into the primate and provide evidence that chemical lesions such as MPTP treatment also may induce this neurite-promoting activity in the brain.

500.12

PROTEASE NEXIN-1, A PROTEASE INHIBITOR WITH NEUROTROPHIC ACTIVITY, IS REDUCED IN ALZHEIMER'S DISEASE. S. L. Wagner*, J.W. Geddes, C.W. Cotman, A.L. Lau*, P.J. Isackson* and D.D. Cunningham* (SPON: R. F. Young). University of California, Irvine, CA 92717.

The biochemical basis for the neuropathological lesions and the loss of cognitive functions that characterize Alzheimer's disease are unknown. The recent findings that brain amyloid deposits of Alzheimer's disease contain trypsin and chymotrypsin inhibitors indicate that an imbalance of proteases and protease inhibitors may be involved (Ponti, P. et al.; Tanzi, R., et al.; Kitaguchi N. et al.; Nature, 331:525-532, 1988). It recently was shown that thrombin, but not other proteases tested, produces retraction of neurites in cultured neuroblastoma cells (Gurwitz, D. and Cunningham, D., Proc. Natl. Acad. Sci. U.S.A., in press). This may be important in neural function since the neurotrophic activity of protease nexin-1 (PN-1) depends on its ability to inhibit thrombin.

The present studies showed that PN-1 activity was eight-fold lower in autopsy brain samples from 10 Alzheimer's disease cases compared to 6 control cases. PN-1 activity was quantitated based on its ability to form SDS-resistant complexes with ^{125}I-thrombin that were blocked by a specific anti-PN-1 monoclonal antibody (Wagner, S.L., Van Nostrand, W.E., Lau, A. and Cunningham, D., Biochemistry, 27:2173, 1988). The much reduced PN-1 activity in the Alzheimer's disease samples was not due to differences in postmortem delay, age or sex. We suggest that the reduced levels of PN-1 in Alzheimer's disease brain could result in increased thrombin levels that in turn could lead to disrupted interactions among neurites and altered neurite morphologies.

500.13

INSULIN INFLUENCES ASTROGLIAL MORPHOLOGY AND GFAP EXPRESSION IN VITRO. C.D. Toran-Allerand, J.P. Anderson* and W. Bentham*. Dept. Neurol./Anat. and Cell Biol., Ctr. for Reprod. Sci., Columbia Univ., P&S, New York, NY 10032.

We report here for the first time that variations in the levels and timing of insulin exposure influence differently the morphological patterns of astroglial differentiation as well as expression of GFAP in long-term organotypic cultures of E-17 mouse cerebellum. The medium was supplemented with no insulin, low insulin (10 pg/ml); or high insulin (10 ug/ml). Cultures were stained immunocytochemically with monoclonal antibodies to GFAP or processed for in situ hybridization, using a biotinylated cDNA GFAP probe. High insulin elicited an increase in GFAP mRNA and intense GFAP immunoreactivity. Low insulin produced minimal expression of message and protein product. The very morphology of GFAP+ cells was also influenced by the hormone concentration in an age-specific manner. Fetal radial glia were selectively increased by high insulin, comprising 64% of GFAP+ cells in contrast to a reversal of this pattern by low insulin, where 69% of GFAP+ cells were flat cells. In newborn cultures, however, the morphological responses to high and low insulin were considerably attenuated. In view of the critical dependence of interactions of developing neurons with radial glia for neuronal migration, differentiation, and the initiation of neurite growth, these changes in morphology suggest developmentally-regulated mechanisms by which insulin-related peptides may influence directly and indirectly neuronal and astroglial differentiation.

500.14

ACTION OF GANGLIOSIDE ON RESPONSIVENESS OF SENSORY GANGLIA TO TROPHIC AGENTS. D.F. Chen* and F.J. Roisen. Dept. of Anatomical Sciences and Neurobiology, Sch. of Med., Univ. of Louisville, Louisville, KY 40292.

Gangliosides potentiate the action of Nerve Growth Factor (NGF) on chick embryonic 9 day (ED) sensory ganglia (DRG). The responsiveness of DRG neurons to NGF begins on ED 7, is maximum on 9 and declines totally by ED 13. To determine if GM1 could alter sensitivity to NGF during developmentally reduced responsiveness, DRG (ED 6-14) were explanted in Medium 199 containing 10% heat-inactivated fetal bovine serum supplemented with GM1 (150 ug/ml) in the presence or absence of NGF (1 ug/ml). Neuritogenesis was evaluated in terms of neurite number and length and by ornithine decarboxylase activity. No GM1 potentiation was observed during the peak of NGF-mediated development (ED 8-10); on other days, treatment with GM1 + NGF enhanced growth. Since medium conditioned over C6 glioma cells for 3 days (GCM) stimulates DRG in the presence of anti-NGF, the ability of GM1 to potentiate GCM-mediated growth was examined (ED 6-14). Maximal potentiation of GCM by GM1 occurred on ED 9-10 when the GCM alone was submaximal. GM1 enhanced the trophic agents when their activity was submaximal by altering the extent but not the duration of the response. These studies demonstrate that gangliosides play a regulatory role in trophic interaction. Supported by NIH NS24524 and DE07734.

500.15

THE NEURITOGENIC ACTION OF PHOSPHATIDIC ACID. W.H.FENG* G. YORKE, K.C. LESKAWA* AND F.J. ROISEN. Department of Anatomical Sciences and Neurobiology, School of Medicine, University of Louisville, Louisville, KY 40292.

Gangliosides can regulate the neuritogenic and neuronotrophic development of several types of embryonic neurons and neuroblastoma in vitro. We have shown that individual gangliosides produce subtle differences in the neuritic patterns. To determine the minimum molecular structure required to produce these changes, we examined the effect of exogenous phosphatidic acid (PA) on neuritogenesis. PA was suspended in phosphate buffered saline, sonicated, diluted with nutrient medium at various concentrations (3.3×10^{-8} to 3.3×10^{-4} M) and applied to Neuro-2a cells. Growth was evaluated in terms of neuritic complexity and % cells with processes. PA was stimulatory over a broad concentration (maximal at 3.0 uM) at 24h. An index of PA's metabolic action was obtained from ornithine decarboxylase induction. Maximal activity was obtained at 30 uM. To determine if PA could potentiate Nerve Growth Factor (NGF)-mediated neuritogenesis, the effect of PA on NGF-mediated development of rat pheochromocytoma PC-12 cells and chick embryonic dorsal root ganglia (DRG) was examined. PA had a moderate effect on PC-12 and DRG differentiation. These data demonstrate that although the responses were not equivalent both PA and GM1 enhance neuritogenesis. Supported by NIH NS24524 and DE07734.

500.16

GANGLIOSIDE AGF2 PROMOTES RECOVERY OF AF64A-INDUCED BEHAVIORAL AND NEUROCHEMICAL DEFICITS. D.F. Emerich, M.J. Spates * and T.J. Walsh Rutgers University, Department of Psychology, New Brunswick, NJ 08903.

AGF2 is the internal ester of GM1 and has been shown to promote recovery of function following central nervous system insult. The studies presented here examined the effects of AGF2 on the behavioral and neurochemical alterations induced by intraventicular administration of AF64A. This cholinotoxin produces long-lasting decreases in the activity of choline acetyltransfase (ChAT) in the hippocampus (HPC) together with persistent cognitive impairments.

Sprague-Dawley rats were trained on a standard eight arm radial maze (RAM) task. Following training, rats were injected (IP) with 10 mg/kg AGF2 or 0.9% saline for 3 days prior to and for 14 days following the bilateral injection of AF64A (3nmol/side/icv.) or artificial CSF. Rats injected with AF64A (AF64A/Sal) were markedly impaired in their performance of the RAM task. In contrast, animals receiving AGF2 (AF64A/AGF2) were initially impaired but rapidly reaquired the task and performed as well as controls.

Rats were then trained to perform a working memory version of the RAM task in which they had to remember which 4 of the 8 maze arms they obtained food from prior to a one hour delay. Following the delay the rats were returned to the maze and allowed to choose freely among all 8 arms. Arms not previously choosen were baited, and entry into previously entered arms constituted an error (delayed-non match-to-sample). AF64A-treated rats, whether treated with AGF2 or not were profoundly impaired on this version of the task and showed no evidence of recovery.

AF64A produced a 35% decrease in hippocampal ChAT activity (AF64A/CSF) that was significantly attenuated by prior treatment with AGF2 (22% decrease). These data suggest that, in this model system, AGF2 promotes recovery of function by either limiting the initial effects of AF64A or by facilitating neural reorganization following the insult.

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500.17

A RAT HYPOTHALAMIC CELL LINE WHICH IS SENSITIVE TO SEVERAL DIFFERENT TISSUE GROWTH FACTORS. I. Torres-Aleman*, F. Naftolin*, and R. J. Robbins (SPON: S. Spencer). Sect. of Neuroendocrinology, Dept. of OB/GYN and Medicine, Yale Medical School, New Haven, CT 06510.

Trophic factors affecting brain cell development appear to be cell-type specific. A single neuron may respond to different growth factors as a function of its developmental state. We examined the possibility that hypothalamic cells may possess responsiveness to more than one growth factor. A stable SV-40 transformed rat embryo hypothalamic cell line (F-12) was grown in the presence of either FGF_b, insulin, IGF-I or MSA (from 0.1 pM to 10 nM). Dose dependent growth promoting effects were present in the following potency: FGF_b>IGF-I = insulin. MSA had no effect. At 0.1 nM FGF_b produced a 20 fold increase in cell number, IGF-I a 10 fold, and insulin a 9 fold. ED_{50} was between 0.1 and 1 nM for all three peptides. We suggest that development of hypothalamic cells may occur in part via regulated or pre-programmed expression of a repertoire of growth factor receptors.

500.18

EFFECT OF NGF ON THE ADULT INTACT AND LESIONED SEPTOHIPPOCAMPAL CHOLINERGIC SYSTEM. M. Fusco*[1], G. Vantini[1], L. Cavicchioli*[1], N. Schiavo*[1], M. Zaremba*[2], M. Gradkowska*[2], B. Oderfeld-Nowak*[2] and A. Leon[1]. [1]Fidia Research Laboratories, Abano Terme, Italy and [2]Department of Neurophysiology, Nencki Institute for Experimental Biology, Warsaw, Poland.

In contrast to neonatal rats, intracerebrally-administered NGF has been reported to significantly affect septal and hippocampal choline acetyltransferase (Chat) activities in adult rats solely following transection of the septal-hippocampal pathway. We here report that continuous intracerebroventricular infusion of NGF (25 μg/2 weeks via mini-osmotic pumps) induces significant increase of Chat activity also in the septohippocampal cholinergic system of sham-operated rats. Furthermore, in rats with unilateral partial fimbria fornix transections, NGF increases Chat activity in the hippocampus of not only the lesioned but also unlesioned side. Whereas the more pronounced NGF effect was observed in the ventral pole of the hippocampus contralateral to the lesion (also in sham-operated animals), the NGF effect was higher in the dorsal pole of the ipsilateral hippocampus. These findings significantly extend the role of NGF in the physiological functioning of adult forebrain cholinergic neurons.

500.19

NGF ENHANCES THE EXPRESSION OF NGF RECEPTOR MESSENGER RNA IN VIVO. L. Cavicchioli*[1], G. Vantini[1], T. Flanigan*[2], F. Walsh[2], M. Fusco*[1], E. Bigon*[1], D. Benvegnù*[1] and A. Leon[1] (SPON: G. Toffano). [1]Fidia Research Laboratories, Abano Terme, Italy and [2]Institute of Neurology, The National Hospital, London, U.K.

Studies of the expression of the NGF receptor in septal cultures have shown that NGF treatment increases NGF receptor immunostaining. In an attempt to study ligand-mediated regulation of NGF receptors in vivo, we assessed NGF receptor mRNA in the septal area of neonatal rats treated daily with an intracerebral injection of NGF (5 μg) or vehicle, from postnatal days (P) 2 to 8. Northern blot hybridization of total RNA extracted from the basal forebrain of each single rat at P9 was carried out by utilizing both cDNA and cRNA probes for human NGF receptor (Chao, M. et al., Science, 232:518, 1988). In addition, choline acetyltransferase (Chat) activity in the striatum and hippocampus of the same animals was assessed to monitor NGF efficacy.

Results show that NGF i) increased both striatal and hippocampal Chat activity (220% and 180% of control, respectively), and ii) caused a marked increase (approx. 3 to 4 fold) in the NGF receptor mRNA levels in the septal area. Densitometric quantification of the latter was normalized by assessment of P1B15 mRNA. Studies are in progress to determine the correlation between the NGF-mediated increase in NGF receptor mRNA and cell surface NGF receptor availability.

501.1

LOCALIZATION OF PROTEIN BINDING SITES IN THE 5' REGION OF THE DYNORPHIN GENE. M.J. Iadarola, J.P. Quinn*[1], J. Douglass*[2] and D. Levens*[1]. Neurobiology and Anesthesiology Branch, NIDR, [1]Laboratory of Pathology, NCI, NIH Bethesda, MD 20892; [2]Vollum Institute, Oregon Health Sciences University, Portland, OR 89230.

Enhanced expression of the dynorphin gene has been observed in several CNS regions during specific types of stimuli. For example, activation of primary afferents or kainic acid seizures cause a marked accumulation of preprodynorphin mRNA in, respectively, spinal cord and hippocampus. These stimulus dependent alterations suggest an increase in dynorphin gene transcription, possibly via factors that bind to regulatory DNA elements. We have examined the 5'end of the rat dynorphin gene for regions that bind such regulatory proteins using a unique binding/exonuclease digestion procedure (Quinn et al Mol Cell Biol, 7:2735, 1987). A 3'end labeled 2,400 base sequence of the 5'region of the dynorphin gene plus the lac operator was tethered, via a lac repressor-beta galactosidase fusion protein, to an acrylamide bead by an antibody bridge. Incubation with crude tissue, whole cell or nuclear extracts allowed binding of proteins to specific recognition sequences. The bead/antibody/DNA/protein complex was separated from the remainder of the extract by centrifugation and digested with T7gene6 exonuclease. At specific sites, digestion was prevented by tightly bound proteins (stop sites). In extracts of rat spinal cord, striatum, cerebellum and 10 other regions electrophoresis revealed 6 prominent stop sites which bracketed the transcription initiation start site. Comparison with peripheral tissues and several cultured cell lines showed one of the sites to be neural specific. Binding of all proteins could be competed by excess unlabeled dynorphin gene but not by poly-dAdT or poly-dIdC or several oligonucleotides. The method provides a rapid procedure to scan large gene fragments for DNA binding proteins which may participate in transcriptional regulation. These results demonstrate the presence of a neural specific protein that recognizes a specific sequence in the dynorphin gene.

501.2

STRONG EVOLUTIONARY CONSERVATION OF NEUROPEPTIDE-Y AND THE CARBOXYTERMINAL PEPTIDE OF THE NEUROPEPTIDE-Y PRECURSOR (CPON). D. Larhammar¶ and R. J. Milner. Research Institute of Scripps Clinic, La Jolla, CA 92037, ¶ and Dept of Med. Genetics, Uppsala University, S-752 45 Uppsala, Sweden.

Neuropeptide-Y (NPY) is a 36-amino-acid neuropeptide which occurs abundantly throughout the mammalian nervous system. NPY forms a family of related peptides with peptide-YY (PYY) and pancreatic polypeptide (PP) which are 70% and 50% homologous to NPY, respectively. The NPY precursor consists of a signal peptide, mature NPY, and a 30-amino-acid carboxyterminal peptide called CPON.

We have deduced the sequence of chick prepro-NPY from gene and cDNA clones. The chick NPY precursor has the same organization as mammalian prepro-NPY. The mature chick NPY peptide has only a single amino-acid difference as compared to the rat and human sequences. To explore the evolutionary conservation of NPY further, Southern hybridizations were performed to genomic DNA of several vertebrate species using a single-exon rat NPY probe. Crosshybridizing fragments were detected in genomic DNA of a reptile, an amphibian, and a bony fish. Thus, NPY is an extremely well-conserved peptide.

Surprisingly, CPON is also highly conserved between chicken and mammals. Chick CPON is 90% homologous to its rat equivalent and 87% homologous to the human peptide. Thus, CPON is as conserved as insulin and VIP. This suggests that CPON has a biological function other than its putative involvement in the processing of prepro-NPY.

The strong sequence conservation of NPY and CPON suggests that these peptides fulfill evolutionarily old and important functions.

501.3

EVIDENCE FOR GENE CONVERSION BETWEEN THE OXYTOCIN AND VASOPRESSIN GENES OF BRATTLEBORO RATS. F.W. van Leeuwen, E.M. v.d Beek, J.P.H. Burbach* and R. Ivell**. Neth. Inst. for Brain Res., *Rudolf Magnus Inst. for Pharmacol., Utrecht, **Inst. for Horm. and Fert. Res., Hamburg, F.R.G.

Propressophysin, consisting of vasopressin (VP), neurophysin (NP) and glycopeptide (GP), is synthetized in about 4500 cells in the rat hypothalamus. In homozygous diabetes insipidus Brattleboro rats (HO-DI) a deletion of a single G residue in the NP-exon constitutes a frame shift mutation. Instead of GP a mutant peptide (MP) is synthetized that cannot be transported. However, a small number (n=20) of GP cells and fibres was found in the hypothalamus and the neural lobe of young HO-DI rats. The most likely explanation for these contradictory results is gene conversion: the oxytocin gene would transfer information to the nearby and deleted VP gene; their strongly homologous NP-exons would be relocated in front of each other by hairpin loop formation enabling mismatch-repair. The normal "reading frame" would be restored and GP synthetized again. Gene conversion means that GP-cells, which express both alleles, have become heterozygous: 1 allel remains coding for MP, the other would express GP. Indeed the same cells displayed both GP and MP immunoreacticity. Furthermore, the number of GP cells increased during life to 95 in 60-week-old HO-DI rats illustrating that gene conversion also occurs in somatic cells. These results show for the first time that long after the last mitosis the genome can undergo changes in the brain.

501.4

CLONING OF RAT CATECHOL-O-METHYLTRANSFERASE (COMT). MH GROSSMAN[1], XO BREAKEFIELD*[2], A FOOTE*[2], K SEAMAN*[3] and CR CREVELING[3] 1-Dept. Peds., Temple Univ. Sch. of Med., 2-Molec. Neurogent., Shriver Ctr., 3-Lab. Bioorganic Chem., NIH-NIAMDD.

To determine the relationship between the different molecular weight and isoelectric forms of rat COMT (EC 2.1.1.6) we have undertaken the cloning of the gene for the rat enzyme. An antiserum to highly purified rat liver COMT was used to screen a rat liver cDNA expression library. A total of 13 positives were identified from two screenings of 84,000 plaques. A frequency of about 0.1%, within the predicted range, was obtained. Several of the larger clones cross-hybridized (E1/M3, and K1/M2/28) and also identified the same size mRNA on Northerns, 1.1 kb and 1.6 kb respectively. All five clones showed hybridization patterns to mRNA from rat tissues consistent with enzyme levels; strongest signals in liver, then kidney, with weaker ones in brain and little or none in heart or spleen. In addition, we made use of an immuno-affinity -purified mRNA, which when translated in vitro coded for a single polypeptide of the size and pI of authentic COMT enzyme. A radiolabeled cDNA probe made from this message was hybridized to a Southern blot of positive clones. All of the larger clones showed signals upon autoradiography.

We are using these clones for in situhybridization to tissues, as well as in screening somatic cell hybrids with human chromosome 22. Ultimate confirmation will be made by comparison of nucleotide and amino acid sequences. Recently, peptide sequence became available. All clones are in M13. We have found sequences responsible for the cross-hybridization of some clones, but no DNA sequence corresponding to the peptide. This work was supported in part by grants from the Scottish Rite Schizophrenia Research Program, and the NIH (NS24066-02).

501.5

CHARACTERIZATION AND REGULATION OF RAT AND HUMAN TYROSINE HYDROXYLASE GENES. G.T. Coker III*, L.B. Vinnedge* and K.L. O'Malley. (SPON: L. Berg) Anatomy and Neurobiology, Washington Univiversity School of Medicine, St. Louis, MO 63110.

Previously, we have characterized the tyrosine hydroxylase (TH) gene from both rat and human sources. In order to understand the functional and evolutionary significance of particular structural features, we have compared introns and flanking DNA between these species presuming important sequences will be highly conserved. The six introns sequenced to date (1,3,4,5,7,8) together with the 3' noncoding region, have identities between 51 and 67%. In contrast, the rat and human TH promoter regions from +1 to -380 bp are 74% identical. We are currently using transient expression assays together with mutational analysis of the promoter region to delineate cis-acting transcriptional sequences for these two genes. When DNA fragments containing these upstream regions are fused to the gene for luciferase both rat and human promoters allow luciferase to be expressed in fibroblasts (3T3) and neuronal cells (PC12). The levels of luciferase produced from these constructs were between 0.5 and 27% the level observed when the rous sarcoma virus promoter was used.

Recently, we have shown that the human TH gene is contiguous with the insulin gene on chromosome 11. To determine whether this is a syntenic phenomenon, we are exploring the linkage of these genes in other species. In rat and mouse the distance between TH and insulin appears to be greater than that in humans (approximately 14-20 kb respectively, versus 2.7). Detailed examination of this unique chromosomal region may provide insights into the gene regulation of both TH and insulin.

501.6

CHARACTERIZATION OF THE RAT OPSIN PROMOTER. M.A. Morabito* and C.J. Barnstable. Rockefeller University, New York, NY 10021, and Dept. Ophthalmology, Yale Univ. Sch. Med., New Haven, CT 06510.

Rhodopsin is the light absorbing pigment of the vertebrate retina. The expression of the opsin gene is restricted to rod photoreceptor cells and is developmentally regulated. Opsin RNA accumulates to detectable levels at postnatal day 2 (PN2) and increases to the adult level at PN10. In parallel, opsin transcripts can be detected at PN1 and the transcription rate increases from PN3 to reach the adult level at about PN10, suggesting that transcriptional regulation is responsible for the increase in opsin expression (Treisman J.E. et al., Mol. Cell. Biol. 8:1570-79, 1988).

The efficient and accurate transcription of eukaryotic protein-coding genes requires *cis*-elements located upstream of the gene, together with *trans*-acting proteins capable of interacting with the promoter regions.

To analyze the role of *cis* and *trans* acting factors in the regulation of the opsin gene, we have isolated clones encoding opsin from a rat genomic library. The nucleotide sequence of the coding region, TATA box and adjacent G-rich region of the rat opsin gene shows 87% homology with the bovine opsin gene previously described (Nathans J. and Hogness D.S., Cell 34,807-814, 1983).

The promotor region has been further characterized by gel retardation and DNA footprinting using nuclear extracts from rat retina, brain and liver tissues. This analysis shows that regions of tissue specific binding are interspersed with regions recognized by ubiquitous proteins. Supported by NIH grants NS 20483 and EY 05206.

501.7

AN UPSTREAM REGION OF THE RAT PNMT PROMOTER CONFERS RESPONSIVENESS TO DEPOLARIZATION IN FUSION GENE CONSTRUCTS. *ME Ross, MJ Evinger, JM Carroll, L Mucke*, SE Hyman, DJ Reis, TH Joh and HM Goodman**, Dept. of Molec. Biol., Mass. Gen. Hosp., Boston MA 02114, Divs. of Neurobio. and Molec. Neurobio., Cornell Univ. Med. Coll., NY, NY 10021.

Neuronal stimuli modulate the expression of phenylethanolamine N-methyltransferase (PNMT) *in vivo*. *In vitro*, we found that bovine adrenal chromaffin cell levels of PNMT mRNA increase (5-8 fold) in response to K^+ depolarization (Neurosci. Abst. 13:1086, 1987). We sought to identify a region of the PNMT promoter which mediates the response to K^+. PNMT 5' flank (1.1 Kb) including the transcriptional start (CAP) site was isolated from a rat genomic library (G. Scherer), probed with rat PNMT cDNA. This fragment was sequenced and the CAP site was determined by primer extension analysis to be 22 bp downstream of the TATA box. PNMT fusion gene constructs with a chloramphenicol acetyltransferase (CAT) reporter were made using pBLCAT2 or pBLCAT3 (B.Luckow). Plasmid pBL_3900 contains the entire PNMT fragment in the promoterless pBLCAT3 while pBL_2500 contains the most distal 480 bp ligated onto the minimal thymidine kinase promoter of pBLCAT2. pBL_3900 was introduced into rat C6 glioma cells with the neomycin resistance marker pRSVneo and stable transformants were selected in G418. Several stable lines consistently displayed a 2-4 fold increase in CAT activity when treated for 16 hrs with 50 mM K^+, compared to matched Na^+ controls. pBL_2500 was cotransfected with pRSVβgal into C6 cells and transients were treated with K^+ or Na^+. CAT values were normalized to β galactosidase activity. The pBL_2500 constructs increased CAT expression 2-4 fold in response to K^+. This suggests that an element responsive to depolarization is present in the region between -400 and -900 bp, relative to the CAP site, of the PNMT promoter.

501.8

cDNA CLONING AND SEQUENCE DETERMINATION OF ARPP-16, A SUBSTRATE FOR cAMP-DEPENDENT PROTEIN KINASE, ENRICHED IN THE BASAL GANGLIA

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ARPP-16 (a phosphoprotein substrate for cAMP-dependent protein kinase of Mr 16,000) has been found to be enriched in the basal ganglia. ARPP-16 has been purified to homogeneity from the supernatant of bovine caudate nucleus and rabbit serum antibodies prepared. An additional substrate for cAMP-dependent protein kinase, with an Mr of 19,000 (ARPP-19), was found to cross-react with the antibodies prepared against ARPP-16. Immunological analysis indicated that ARPP-16 was enriched in the basal ganglia while ARPP-19 was present in similar levels in all brain regions studied and was also present in non-neuronal tissues. cDNA clones were isolated from a bovine caudate cDNA library using in situ colony hybridization with oligonucleotide probes designed on the basis of the amino acid sequences of several peptides purified from chymotryptic digests of ARPP-16. Two distinct cDNA clones were isolated and the nucleotide sequences determined. Comparison of the nucleotide sequences and amino acid sequences indicates that one clone codes for ARPP-16 while the other codes for ARPP-19. The amino acid sequences of ARPP-16 and ARPP-19 are identical except that ARPP-19 has an additional 16 amino acids at the N-terminus. The two cDNA clones share an identical 3' untranslated region of 756 nucleotides. In addition, the cDNA clone for ARPP-16 contains 806 nucleotides following the common sequence. The 5' untranslated regions of the two clones are entirely different. These results suggest that ARPP-16 and ARPP-19 may be produced by tissue- and brain region-specific, alternative splicing of a primary transcript.

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501.9

ISOLATION AND CHARACTERIZATION OF GENES SPECIFICALLY EXPRESSED IN THE ELECTROMOTOR NUCLEUS OF TORPEDO.

M. Linial*, J. Ngsee*, and R.H. Scheller. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

Polyclonal antibodies raised against purified cholinergic synaptic vesicles from Torpedo californica were used to screen a λgt-11 expression cDNA library constructed from the electromotor nucleus (Carlson, S.S., and Kelly, R.B. (1980) J. Cell. Biol. 97, 98-103). Three of the cDNA clones were shown to be specifically expressed in the electromotor nucleus and not in the electric organ, gill, muscle, heart, skin or liver. Moreover, the level of expression is approximately 10 fold higher in the electric lobe relative to the brain. The cDNAs hybridize to three independent mRNAs of 10 kb (clone 2), 5.5 kb (clone 3) and 2.0 kb (clone 4). Searches of the protein data bank using amino acid sequences predicted from the nucleotide sequences of the cDNAs did not reveal any significant homologies. Antibodies raised against the coding region of clone 2 recognize a unique protein with an apparent molecular weight of 350,000 daltons which copurifies with Torpedo synaptic vesicles.

VISUAL CORTEX VII

502.1

CORTICAL DYNAMICS OF FORM AND COLOR PERCEPTION. S. Grossberg and E. Mingolla*. Center for Adaptive Systems, Boston Univ., Boston, MA 02215.

A model cortical architecture is described for explaining key processes of boundary detection, sharpening, regularisation, and completion; textural segmentation; shape-from-texture; and filling-in of brightness and color. The architecture clarifies the functional role of reciprocal interactions among the hypercolumns, blobs, and stripes of areas V1, V2, and V4, and of the cell types: simple, complex, hypercomplex, opponent, and double-opponent. The model characterises two functionally complementary cortical systems: A Boundary Contour System (BCS) and a Feature Contour System (FCS). The first stage of BCS (FCS) is at striate hypercolumns (blobs). The BCS controls the emergence of 3-D segmentation of a scene. The outcome of this 3-D segmentation process is perceptually invisible within the BCS. Visible percepts are a property of the FCS. A completed segmentation within the BCS elicits topographically organised output signals to the FCS. These completed BCS signals regulate the hierarchical processing of color and brightness signals by the FCS. Notable among FCS processes are the extraction of color and brightness signals that are relatively uncontaminated by changes in illumination conditions. These Feature Contour signals interact within the FCS with the output signals from the BCS to control featural filling-in processes. These filling-in processes lead to visible percepts of color-and-form-in-depth at the final stage of the FCS at V4. The model has been used to explain and predict a large body of psychophysical and neural data, such as data of Peterhans and von der Heydt on V1–V2 interactions involved in completion of illusory boundaries, Desimone *et al.* on multiplexing within V4 receptive fields, Srebo and Osetinsky on prestriate generators of vernier acuity, Spitzer and Hochstein on complex cell receptive fields, and Livingstone and Hubel on color processing.

502.2

MAXIMUM-INFORMATION PRESERVATION: A PROPOSED ORGANIZING PRINCIPLE FOR CERTAIN ASPECTS OF PERCEPTUAL NEURAL ARCHITECTURE. R. Linsker. IBM T. J. Watson Research Center, Yorktown Heights, NY 10598.

What principles might account for the strikingly complex sets of feature-analyzing properties found in mammalian perceptual systems, and for their organization and integration?

A Hebb-type synaptic modification rule causes model cells in a feedforward network to develop so that (under certain conditions) each cell's output activity conveys maximum information about its input activity values [R. Linsker, Computer 21(3): 105 (March 1988); see also Proc. Natl. Acad. Sci. USA 83: 7508, 8390, 8779 (1986)].

This suggests a potential organizing principle, 'maximum information preservation,' for each processing stage of a multilayered perceptual network having feedforward and lateral (intralayer) connections. According to this principle, each processing stage develops so that the output signal values (from that stage) jointly convey maximum information about the input values (to that stage), subject to certain constraints. (The quantity that is maximized is a Shannon information rate.) This principle may be implemented by activity-dependent mechanisms (of which a Hebb-type rule can be a part) and/or other developmental mechanisms.

For certain simple ensembles of input activities, this principle generates topographic maps, 'cortical magnification' effects, and layers of feature-analyzing cells that extract and encode statistical regularities present in their input environment (in spatial, temporal, acoustic frequency, optical wavelength, and other domains). Some extensions of these results to input ensembles and networks of biologically relevant complexity are presented and compared with experimental findings in visual cortex.

502.3

SUDDEN COLOR-BLINDNESS OF CEREBRAL ORIGIN. O. Sacks*, R.L. Wasserman*, S. Zeki* and R.M. Siegel Albert Einstein College of Medicine, New York City, NY 10461, Southhampton Hospital, Southhampton, NY 11968, University College, London, U.K. and The Rockefeller University, New York City, NY 10021.

Achromatopsia of cerebral origin was described a century ago and found to be associated, at autopsy, with vascular or other lesions of visual cortex. Such lesions have also been demonstrated in life by neuro-imaging. The majority of such patients also have visual field defects, visual alexia and some degree of form agnosia (Pearlman et al., 1979; Damasio, 1980). Pure achromatopsia is rare. The patient studied by us- a painter-is unusual in combining a complete achromatopsia with an exceptional prior sensibility for color. The achromatopsia, which followed the onset of a transient alexia, came on suddenly following a head injury. There was a complete loss of color throughout the visual field, the patient seeing only in varying shades of grey (the world appeared "as if molded in lead"). The patient, who had exceptional powers of visual imagery and recall, now found that his recalled images were equally void of color. With color perception denied him, he was forced to paint in black and white. Saturated reds and greens both looked black to him; but saturated blues looked very pale. This was even the case with isoluminant Farnsworth-Munsell buttons. His grey-scale was also impoverished, but his lightness scale at all wavebands was unaffected. Recognition was difficult in low-contrast situations. There was apparently no form-, depth- or movement-agnosia, and no defects in the visual fields (at least when seen by us ten weeks after his injury). More formal testing with color 'Mondrians' (Land, 1977) suggested that wavelength-discrimination was intact, but color "construction" entirely absent. This suggests that damage is confined to the "color coding" areas of pre-striate cortex homologous to V4 (Zeki, 1973). The apparent isolation of J.I.'s achromatopsia is in good agreement with current understanding of segregation of different visual functions with separate cortical "functional systems" (Zeki, 1978). Further psychophysical and anatomical tests are in progress to determine the precise site of damage.

502.4

THE "MOTION-BLIND" PATIENT: A PSYCHOPHYSICAL STUDY OF SPATIAL AND TEMPORAL VISION. R.F. Hess*, C.L. Baker (Jr.), and J. Zihl*. Physiological Laboratory, University of Cambridge, Cambridge, England; Psychology Dept., McGill Univ., Montreal, Canada; and Max-Planck-Institut for Psychiatry, Munich, F.R.G.

Zihl et al (1983) have described a "motion-blind" patient, who had suffered a specific loss of visual motion perception resulting from a vascular failure. Other visual functions, such as acuity, color vision, critical flicker fusion frequency, and stereopsis, were normal. We have used discrimination psychophysical methods and sinewave grating stimuli to further characterize this patient's deficit.

Contrast sensitivity to detect the presence of a grating, as a function of its spatial and temporal frequency, was found to be only slightly impaired. However contrast sensitivity to discriminate the direction of motion of a drifting grating showed severe reduction.

For suprathreshold gratings, contrast discrimination and spatial frequency discrimination were only slightly affected, even if the gratings were moving. However, temporal frequency or velocity discrimination of these gratings was severely impaired.

These findings are consistent with an extrastriate locus of damage.

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502.5

NEURAL DETERMINATION OF THE DIRECTION OF MOTION: CONTRAST AFFECTS THE PERCEIVED DIRECTION OF A MOVING PLAID.

L S Stone, J B Mulligan*, and A B Watson*, Vision Group, NASA, Ames Research Center, MS 239-3, Moffett Field, CA 94035.

Plaids, the sum of sinusoidal gratings of different orientations, have been useful in studying how the primate visual system determines the motion of an object as a whole (pattern motion) from information about the motion of oriented components within that object (component motion). The intersection of the constraints imposed by the motion of the two grating components within a plaid (the constraints rule) provides a reasonable qualitative estimate of psychophysical results. Neurons which respond selectively to pattern motion have been found in the middle temporal area of extrastriate visual cortex suggesting that the neural determination of pattern motion may occur there [1]. By examining how the relative contrast of the grating components affects the perceived direction of pattern motion, we hope to shed light on the underlying neural processing.

We made quantitative measurements of the perceived direction of motion of plaids whose component gratings were of different contrasts. Human subjects viewed moving plaids composed of two achromatic luminance gratings of equal spatial frequency (1.5 cyc/deg), orientated symetrically 30 deg off the vertical axis. In each trial, the plaid moved for 300 ms, at 2 deg/s. The direction of motion of the plaid varied from trial to trial but its orientation did not. Subjects were asked to judge whether the plaid had moved to the left or to the right of vertical. Perceived vertical (the direction for which subjects randomly chose left or right) varied substantially and systematically with the contrast ratio of the grating components. We conducted experiments using different total contrasts (0.1, 0.2, and 0.4) and determined that, at the two lower contrasts, the deviation from the constraints rule (up to 20 deg) was a fixed function of the contrast of the weaker grating relative to its detection threshold. Our results are consistent with schemes in which the estimated speed of the components is subject to a contrast-dependent distortion before application of the contraints rule, and represent an important restriction on future models of the neural processing of motion within visual cortex.

1. Movshon, Adelson, Gizzi, and Newsome, Exp Brain Res Suppl 11:152 (1986).

502.6

THE OPTIMAL DISPLACEMENT FOR THE DETECTION OF MOTION. Jane C. Boulton* and R.F.Hess* (SPON: B. Gordon-Lickey). Physiol. Lab., Cambridge Univ., Cambridge, CB2 3EG, U.K.

Recordings from Cat striate cortex (Baker and Cynader, 1986) have indicated that direction selective cells give an optimal displacement (Dopt). To investigate whether a Dopt could be determined for human motion processing using psychophysical techniques, velocity discrimination thresholds were measured.

Drifting sinewave gratings were used with a method of constant stimuli to obtain velocity discrimination thresholds as a function of stimulus duration for a range of velocities and spatial frequencies. The predicted results on the basis of the above physiology would be that this function should be non-monotonic, exhibiting a local minimum corresponding to an optimum displacement. For stimuli of different spatial frequency a Dopt rule should emerge which can be expressed in terms of a certain fraction of a spatial cycle.

Our results show that there are indeed local minima in the velocity discrimination function and that the Dopt rule that emerges corresponds to 1/6 of a spatial cycle or integral multiples of this. This is in agreement with the single cell results cited above.

502.7

HUMAN PERCEPTION OF 3-D STRUCTURE FROM MOTION: SPATIAL AND TEMPORAL CHARACTERISTICS.

S. TREUE, M. HUSAIN and R.A. ANDERSEN, Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

We have investigated the ability of humans to detect 3-D structure from motion using a reaction-time task. The structured stimulus was created by the parallel projection of random points from the surface of a rotating cylinder. Points lived for a fixed number of display frames (point lifetime). Subjects were required to detect the change from an unstructured stimulus to a structured one.

For the range 8-128 projected points, we found that under optimal conditions the threshold for any reliable detection of change to a structured stimulus was ~40-60 ms point lifetime (~3-4 frames at 70 Hz presentation). For point lifetimes >80 ms (i.e., well above threshold), detection was made more reliable if a large number of points were used. Detection also depends upon other parameters. By increasing the angular rotation rate to $>140^{o}\ s^{-1}$, it was possible to obtain 100% hit rates even when only 8 points were displayed, i.e., there is a trade-off between angular velocity and the number of points. Thus at least three factors interact to influence the perception of structure from motion in humans: the number of points projected, the point lifetime and the angular velocity of the stimulus.

502.8

COMPARISON OF TWO- & THREE-DIMENSIONAL STRUCTURE FROM MOTION: REACTION TIMES & GLOBAL TEMPORAL INTEGRATION.

M. HUSAIN, S. TREUE and R.A. ANDERSEN, Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

In previous experiments, using techniques described in the accompanying abstract, we found shorter reaction times on a 2-D rotation task in comparison to a 3-D cylinder task. Two possible explanations are: i) additional direction cues in the 2-D stimulus, or ii) additional processing time required for the 3-D percept.

To determine between these two, we constructed a stimulus without additional direction cues. It is identical to the 3-D stimulus with the exception that the change in speed occurs orthogonal, rather than parallel, to the direction of movement. This new stimulus elicits a 2-D shear motion percept. Reaction times for the 2- and 3-D tasks were essentially the same over the entire range of point lifetimes and velocities tested. These results suggest that the computation of 2- and 3-D structure from motion occurs in a similar fashion and both sorts of stimuli may be analysed in the same brain region(s).

For both 2- and 3-D stimuli, perceived velocity was greater with shorter point lifetimes. This effect could be compensated for by altering the velocity of the stimulus. Interestingly, flashing, *stationary* random dots within the stimulus were not only "captured" by the moving ones but they also increased the apparent velocity of the entire stimulus. Thus the temporal characteristics of stationary dots are integrated into the computation of the global velocity field.

502.9

CONTRIBUTION OF DIFFERENT PATHS OF THE CAT VISUAL SYSTEM TO DETECTION PERFORMANCE: SINGLE CELL RECORDINGS AND BEHAVIOURAL EXPERIMENTS. W. Kiefer*, K. Krüger*, A. Groh* HRO. Dinse, G. Berlucchi* (Spons. EBBS) Dept. of Zoology III Biophys. Sect., University of Mainz, Saarstr. 21, D-6500 Mainz, West Germany

The cat's visual cortex has more than a dozen distinct areas with input via different centripetal pathways. We investigated the role of the two major pathways using a behavioural approach as well as single cell recordings. Simple geometrical patterns respectively moving bars (signal) were additively superimposed on Gaussian visual noise and the detection probability (P_D) was measured as a function of the signal-to-noise (S/N) ratio for various stimulus conditions either i.e. moving or keeping the signal and/or the background stationary. The highly interconnected system was decoupled by lesioning cortical areas in various combinations and the pre- and postoperative detection curves were compared with the electrophysiologically obtained S/N-thresholds.

The role of the geniculo-cortical system was investigated with bilateral lesions of areas 17/18, area 19, areas 17/18/19, and the role of the extra-geniculo-cortical system with bilateral lesions of the areas of the lateral suprasylvian sulcus (LSA), area 7, and areas 7/LSA.

Four major conclusions may be drawn on the basis of the currently available data: 1. The geniculo-cortical system seems highly involved in extracting patterns as such out of a structured background. 2. The extrageniculo-cortical system seems highly involved whenever patterns are associated with motion. 3. All deficits, with the exception of the large lesion of areas 17/18/19, could not be compensated even by extensive retraining. 4. This last finding is taken as evidence that the structures we investigated have redundant capacities only in a very restricted sense.

502.10

VISUAL REACTION TIME OF THE CAT AS A FUNCTION OF SPATIAL FREQUENCY. M.S. Loop and B. E. Aiken*. Dept. of Physiological Optics, School of Opt., Univ. of Alabama at B'ham., B'ham, AL 35294.

Cats were trained to respond quickly to the presentation of vertical sinewave gratings for a food reward. At 50% contrast, reaction time increased systematically with increasing spatial frequency (0.25, 0.50, 1.0, 1.5, 2.0 cpd). At 0.25 and 2.0 cpd reaction time increased as contrast decreased and reaction time was again faster for 0.25 cpd than 2.0 cpd when sensitivity differences were taken into account. Enthusiastic to attribute detection of 0.25 cpd to Y-cells and 2.0 cpd to X-cells, yet certain to meet equally enthusiastic resistance to this interpretation, we measured reaction time to 50% contract 0.25 and 2.0 cpd surrounded by a large area of 100% contrast flicker at 7 cps or 70 cps. Reaction time to 0.25 cpd was reliably slower when surrounded by 7 cps flicker than when surrounded by 70 cps flicker (which is above the cats CFF and would therefore appear steady). Surround flicker rate had no effect upon reaction time to 2.0 cpd. So whatever visual neurons detect 0.25 cpd and 2.0 cpd the former are affected by stimulation peripheral to their receptive fields while the latter are not. Supported by EY05576.

502.11

TEXTURE DISCRIMINATION AND PERCEPTION OF COGNITIVE CONTOURS IN CATS BEFORE AND AFTER CORTICAL ABLATIONS. S. Aglioti*, G. Berlucchi, M. Corbetta *, A.Antonini * Institute of Human Physiology, University of Verona, I-37134

Four cats were tested on texture discriminations using stimuli differing on some of the conspicuous local features called textons by Julesz. They were able to discriminate such textures and to tranfer this ability immediately to new textures built up by elements never seen before. The above capability survived a bilateral removal of areas 17 and 18. We have also studied the discrimination of figures delimited by the so-called Kanisza's cognitive contours which are perceived by humans in spite of being physically inexistent. The positive stimulus was a cognitive square contour tested against a visual noise constituted by a random arrangement of elements that if ordered in space give rise to the perception of the cognitive contour. Learning required a large number of trials, but the discrimination was retained after bilateral ablation of areas 17 and 18. Moreover, one of the four cats was tested post-operatively on a discrimination between a new cognitive square and a cognitive diamond, or a stimulus built up of the same elements of the cognitive square, disposed at the four angles of a square, but rotated so as to make the cognitive contour disappear. Learning required comparatively few trials, suggesting some degree of transfer from previous experience.

502.12

THE TEMPORAL DYNAMICS OF BINOCULAR RIVALRY: PSYCHOPHYSICAL SUPPORT FOR A RECIPROCAL INHIBITION MODEL. T. J. Mueller and R. Blake*. *Harvey Mudd College*, Dept. of Biology, Claremont, CA 91711 and Dept. of Psychology, *Vanderbilt University*, Nashville, TN 37240.

We present evidence that binocular rivalry responds to stimulus contrast in two ways. 1) The duty-cycle of dominance and suppression is determined by the relative image contrast between the two eyes, with dominance of the higher contrast image being favored, and 2) the overall rate of alternation is driven by monocular image contrast during the suppressed phase (increased monocular contrast increases the alternation rate) and to a lesser extent by monocular contrast during the dominant phase (increased monocular contrast decreases the rate).

These results were obtained with two experimental conditions. Human subjects viewed dichoptic orthogonal sine-wave gratings and indicated when exclusive visibility occurred in either eye. Contrast was held constant in one eye and was increased or decreased in the other eye for a number of alternation cycles (continuous presentation) or for only the duration of a single period of exclusive visibility (synchronous presentation). The synchronous presentation condition allowed us to identify the differing effects of contrast during the suppressed and during the dominant periods.

These results support a reciprocal inhibition oscillator as the underlying mechanism of binocular rivalry. (Supported in part by NSF grant BNS 8418731.)

502.13

COMPUTATIONAL AND SAMPLING CONSIDERATIONS IN THE ANALYSIS OF LOCALIZED AND DISTRIBUTED PROCESSES BY ONE DIMENSIONAL CURRENT SOURCE DENSITY TECHNIQUES. C.E. Tenke, C.E. Schroeder, J.C. Arezzo and H.G. Vaughan, Jr. Depts. of Neurosci. and Neurol., Albert Einstein Coll. of Med. Bronx, NY.

Current source density (CSD) techniques are useful for the identification of the intracranial generators of surface recorded potentials and for the characterization of intracortical processes. Profiles derived from multichannel electrodes with fixed intercontact spacing improve the CSD estimate over sequentially obtained profiles, without requiring spatial smoothing, by eliminating interrecord variability. While localized generators are reliably resolved by these techniques, there has been no analysis of the influence of spatial resolution on the CSD profile. These influences were modelled on a computer using spherical generator elements arranged in dipole and "closed field" configurations and spatially distributed to simulate variations in the dimensions of laminae and columns. Continuous, time invariant field potential profiles were simulated and discretely sampled. CSD profiles were calculated using a common 5-point CSD formula and a 3-point formula with two distinct differentiation grids.

Artifacts introduced into the CSD profile by computational and sampling procedures were more complex for the 5-point method, and were attenuated by increasing the radial area of the generator. While field and CSD profiles emphasized laminar boundaries, artifacts within a lamina could be eliminated with appropriate intercontact separations. Artifacts produced by narrow, radially distributed dipole laminae were shown to have a negligible effect on the descriptive and localizing capacity of the CSD. These results support the use of high resolution, one-dimensional CSD techniques for describing both distributed and localized cortical generators. Supported by MH06723

502.14

SEQUENCE AND DISTRIBUTION OF PATTERN-EVOKED ACTIVITY IN AREA 17 OF THE BEHAVING MONKEY. C.E. Schroeder, C.E. Tenke, S. Givre, J.C. Arezzo and H.G. Vaughan, Jr., Depts. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Using 16 channel, multicontact electrodes, laminar profiles of pattern visual evoked potentials, current source density and concomitant multiunit activity were recorded from striate cortex in fascicularis monkeys, performing a fixation task. Stimulation consisted of chromatic (red/green) and achromatic (black/white) patterns, varying in luminance and pattern content, modulated independently of the fixation point. The laminar response profile, in general, reflects initial activation in the granular (stellate) laminae, followed by activation of supra- and infragranular (pyramidal) laminae. While this sequence conforms to that predicted by anatomic studies, the laminar weighting of activity within the profile varies with the luminance, chromatic and pattern characteristics of stimuli. The profile of response to diffuse illumination is heavily weighted toward lamina IV, with moderate responses in infragranular laminae. Patterned stimuli shift the balance of this profile away from lamina IV. Increasing the chromatic content of stimuli enhances the response in lamina IVCb, relative to IVCa, and in the supra- relative to the infragranular laminae. Quite often our techniques are able to resolve orientation preference in the infragranular laminae, while unable to do so in the supragranular laminae. This, along with the differential sensitivity to diffuse illumination in infra- and supragranular laminae, supports the view that orientation sensitivty operates differently above and below lamina IV.

Supported by MH06723

502.15

THE EQUIVALENT DIPOLES OF TEMPORALLY MODULATED LUMINANCE RESPONSES. P.Ossenblok*, D.Reits* and H.Spekreijse* (SPON: A.B.A. Kroese). The Netherlands Ophthalmic Research Institute, P.O. Box 12141; 1100 AC Amsterdam-Zuidoost, The Netherlands.

Steady state Evoked Potentials (EPs) in response to sinusoidally modulated light can be described and analyzed in terms of frequency selective processes. These frequency selective processes have amplitude peaks in three distinct frequency regions,the so-called low-, medium- and high- frequency region at about 10, 20 and 40 Hz (Spekreijse et al, Visual Evoked Potentials in man: new developments, J.E.Desmedt (ed), Oxford University Press, 1977).

This study deals with the topographical distribution and the location of the neuronal generators of luminance EPs in man, of which the harmonics are plotted as equipotential maps. The analysis is based on the assumption that in each distinct frequency-region the potential distribution of the underlying cortical activity can be ascribed to a single current dipole. A precondition for computing an equivalent dipole from topographic maps is that the phase of the harmonics in all derivations is the same; only phase changes of 180 degrees are allowed.

In correspondence with earlier findings we could confirm that the source of the evoked activity in the high-frequency region is located in the primary visual cortex and that this activity can be described by an equivalent dipole. Further, we found that the recorded potential distribution in the medium-frequency region also can be ascribed to a single current dipole. This equivalent dipole shows a similar behaviour as the one representing the high-frequency subsystem. The orientation of the dipole is radial for foveal stimululation and becomes tangential with increasing stimulus eccentricity. The activity evoked in the low- frequency region is not specific and the source seems to be situated deep in the head.

502.16

AN ANISOTROPIC MULTI-SPHERE MODEL FOR SOURCE LOCALIZATION BY VEPs IN MAN. J.C. de Munck*, B.W. van Dijk and H. Spekreijse* (SPON P.A. Apkarian). The Netherlands Ophthalmic Research Institute, POBox 12141, 1100 AC Amsterdam ZO, The Netherlands.

When multi-channel visually evoked potentials (VEPs) recordings are used for the localization of brain activity mathematical models are needed that describe the electrical properties of the head. Generally it is described as a volume conductor with a piecewise constant conductivity.

None of the present models take into account the anisotropy of the various parts of the head, although anisotropic conduction has been demonstrated for various parts of the head. The anisotropy ratio can amount to a factor of ten for the skull and the white matter, and more than a factor of two for cortical tissue. Further, the present analytic solutions of volume conductor model are restricted to a limited number of shells (four) and a spherical geometry.

It is clear that the use of a simple model may lead to systematic errors in the source localization procedure. Therefore a more general solution has been found, which includes the effects of anisotropy, the arbitrary number of shells and the fact that the head may better be described by a spheroid than a sphere. The solution was obtained by generalyzing the method of Morse and Feshbach to obtain a Greense function of the Laplace equation. The potential distribution due to a dipole source is then found by taking the gradient of the Greense function and determining the inner product of the result and the dipole vector. It is possible to present the formulas in a convenient form, such that if they are applied in practice the likelihood of software errors is minimal.

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502.17

VEP EVIDENCE OF FUNCTIONAL DIFFERENCES IN UPPER AND LOWER VISUAL FIELDS IN MAN. Fred H. Previc. USAF School of Aerospace Medicine, Brooks AFB, TX.

This study investigated differences in the functional characteristics of two components of the pattern-reversal visual evoked potential (VEP) in humans: N1 (which is elicited primarily by upper visual field stimulation) and P1 (which is largely generated by lower visual field stimulation). The stimulus parameters which were used to isolate the two components were grating spatial frequency (1, 4, 8, 12 c/deg) and contrast (.1, .2, .4, .8). VEPs were recorded monopolarly from eight subjects using O_z as the source electrode site. Square-wave gratings, counterphased at 1 Hz, were used to generate each VEP. The gratings were presented at a mean luminance of 15 cd/m^2 and were contained in full-field as well as hemifield presentations. The VEP data revealed the existence of bandpass spatial tuning and little contrast saturation in the case of N1 and lowpass spatial tuning and a nonlinear contrast response (i.e., saturation at high contrasts) for P1. In general, N1 may be viewed as largely reflecting parvocellular processing in the visual cortex, whereas P1 primarily reflects magnocellular output. Thus, these VEP findings parallel recent anatomical and physiological asymmetries (Burkhalter, Felleman, Newsome, and Van Essen, Vision Research, 1986, 26, 63-80) in the processing of information in the upper and lower visual fields.

502.18

PATTERN VISUAL EVOKED POTENTIALS FROM CHILDREN UNDER GENERAL ANESTHESIA. B.E.S. Fox, K.W. Wright*, and K.J. Eriksen*. Univ. of Southern California School of Medicine and Childrens Hospital of Los Angeles, Dept. of Ophthalmology, Los Angeles, CA, 90054.

Pattern visual evoked potentials (PVEPs) were recorded from 9 normal eyed subjects, 9mo-10yrs of age, under general anesthesia. Children were anesthetized, by tracheal intubation, with either Halothane (0.6%) or Forane (1%) and nitrous-oxide for elective surgery unrelated to this study. Silver-silver chloride scalp electrodes were mounted Oz, Cz and Fz. The eye was held open by the experimenter, vision was corrected and fixation was monitored continuously. Alternating (2Hz) black-white checkerboard stimuli with check sizes ranging from 55 to 4 minutes of arc (ma) were used. Transient evoked responses were digitized and averaged. A minimum of 100 trials were obtained for each check size. In two subjects we were unable to obtain PVEPs due to the presence of alpha waves. Results from the remaining 7 subjects revealed that the mean P100 latency increased from 121msec with 55ma checks to 186msec with 14ma checks. Mean P100 amplitudes decreased from 38.1uV to 27.8uV over the same stimulus range. The mean visual threshold obtained was 8.75ma. In general, the waveforms obtained had a broad peak. Four children sedated with chloral hydrate showed PVEPs with similar latencies but smaller amplitudes than were obtained under anesthesia.

These results suggest that PVEPs can be reliably obtained to small spatial frequency stimuli under anesthesia, and may be important in the clinical visual assessment of children.

502.19

HUMAN AND RAT VISUAL EVOKED POTENTIALS IN RESPONSE TO STIMULUS SPATIAL AND TEMPORAL FREQUENCY. W.K.Boyes and H.K.Hudnell. Neurotoxicol. Div., USEPA, RTP, NC 27711.

Species comparisons can indicate the extent to which visual systems are functionally similar and demonstrate the adequacy of experimental animal models. We measured steady-state visual evoked potentials in rats and humans in response to manipulation of stimulus parameters. Spatial frequency of sine-wave gratings spanned 0.05-0.8 cycles/degree (cpd) for rats and 0.5-8.0 cpd for humans; temporal frequency of on/off square-wave modulation spanned 3-20 Hz; and contrast was 20%. Spectral amplitude of the averaged responses was measured at one (1F) and two times (2F) the stimulus rate.

The maximum 1F amplitudes usually occurred at intermediate spatial frequencies approximating the respective contrast sensitivity function peaks of both rat and human. The 2F amplitude was usually maximal at low spatial frequencies. At higher temporal frequencies (10-20 Hz), both species exhibited a drop in 2F and an increase in 1F amplitude, apparently reflecting a response transition from 2F to 1F.

In summary, (1) rat and human visual systems responded in a qualitatively similar fashion to spatial and temporal stimulus manipulations, and (2) 1F and 2F showed different spatial and temporal profiles suggesting contributions of functionally distinct generators. (HKH supported by National Research Council Research Associateship).

502.20

LATE NEGATIVE WAVE OF RAT FLASH EVOKED POTENTIALS: EFFECTS OF TESTING CONDITIONS ON AMPLITUDE. R.S. Dyer, Neurotox. Div., U.S.E.P.A., Research Triangle Park, NC 27711.

A prominent feature of flash evoked potentials (FEPs) recorded from rat visual cortex is a late negative wave with a peak latency of about 155 msec (N155). Depression of N155 amplitude occurs following treatment with a variety of toxic and pharmacological agents. This report focuses upon the relative importance of changes in behavioral state (habituation to the test environment) and stimulus parameters (flash rate and background illumination) as determinants of N155 amplitude. All studies were performed upon unanesthetized adult male Long-Evans hooded rats with previously implanted electrodes overlying visual cortex (VC). Four flash rates (0.5, 1.0, 2.0 and 4.0Hz) and two background illumination levels (0 and 115 lux) were studied. Habituation was investigated by varying the time in the test chamber before testing (1, 2, 4, and 8 min) and by repeated testing (8 test sessions over 3 wks). Amplitudes were measured from baseline. The most critical determininant of N155 amplitude was number of test sessions. As number of 64 trial sessions increased, so did N155 amplitude, which approached asymptote by the final session.Flash rate did not influence, and testing in the dark slightly reduced N155 amplitude. We conclude that although the N155 peak is elicited by a sensory stimulus, its amplitude depends more upon the behavioral state of the animal (i.e. habituation to the testing) than upon stimulus parameters.

503.1

SELECTIVE NEURONAL DEATH IN GERBIL SENSORY NEOCORTEX AFTER TRANSIENT ISCHEMIA. C.S.Lin, B.J.Crain, K.Polsky, J.V. Nadler and J.Davis. Depts. Neurobiology, Pathology, Pharmacology and Medicine,Duke Univ. Med. Cntr., Durham,NC 27710

We used cresyl violet,hematoxylin-eosin/Luxol fast blue,silver impregnation and immunostaining of the glial marker-GFAP methods to assess the selectivity of neuronal death in gerbil neocortex after varying both the duration of bilateral common carotid occlusions and the post-ischemic survival time. A specific laminar pattern was consistently found in the post-ischemic somatosensory (SM) cortex. Six hours after a 5 minute occlusion, fibers and a few scattered small- to medium-sized degenerating pyramidal cells were found in lower layer III and upper layer VI. After the following two days, neuronal death in these layers spread from the facial vibrissae representation area to other areas within SM cortex. This basic laminar pattern persisted up to five weeks (the longest time studied). Occasionally, degenerating cells and fibers were found in the auditory cortex but not in the motor or visual cortex. The pattern of GFAP reactivity also corresponds with this laminar selectivity. Finally, degenerating cells were found in all layers of cortex including the large pyramidal cells in layer V after 15 minutes occlusions. Supported by NIH NS06233.

503.2

SPATIAL MEMORY PERFORMANCE OF GERBILS FOLLOWING TRANSIENT GLOBAL ISCHEMIA. J. P. Williams and J. N. Davis (SPON: J. Hall). V. A. and Duke Univ. Medical Centers, Durham NC 27705.

We used a Morris water-maze to determine if gerbils used spatial memory to solve the maze and if gerbils developed a defect in spatial memory after transient forebrain ischemia. Animals were given a hidden platform that was either stationary (PLACE) or moved with each trial (RANDOM). Four trials were given each day and the latencies in seconds for the animal to find the platforms were recorded. Latencies for both groups decreased with repeated trials ($p<0.0001$). After the 12th trial, the PLACE animals had significantly lower latencies than the RANDOM animals ($p<0.03$). PLACE animals repeatedly crossed the spot where the platform had been ($p<0.001$) when the platform was removed, while RANDOM animals did not swim in any preferred quadrant ($p=0.47$). To study the effect of ischemia, gerbils were divided into 3 treatment groups: ISCHEMIC (5 min carotid occlusion, testing 7 days later), CONTROL (sham surgery or no surgery), and RANDOM (no surgery, random platform placement). All groups showed decreased latencies with repeated trials. The RANDOM group had longer latencies in the last four trials then the two groups with fixed platform placement ($p<0.05$). There was no difference between the final latencies of the ISCHEMIC and CONTROL groups. These data show that: 1) gerbils are capable of solving the Morris water-maze, 2) gerbils use spatial memory to escape to a hidden platform and 3) CA_1 damage in the gerbil does not disrupt this performance. There are two possible explanations for our findings: CA_1 may not be necessary to solve the maze, or the hippocampus may not subserve spatial memory in the gerbil. We prefer the hypothesis that CA_1 is not required for solving the maze.

(Supported by the V.A. and NIH, NS06233)

503.3

CHANGES IN SOMATOSENSORY EVOKED POTENTIALS DURING GLOBAL BRAIN ISCHEMIA IN THE RAT. E.I. Pinkhasov*, A.J. Krieger* and H.N. Sapru (SPON: R. Howland). Section of Neurosurgery, UMDNJ - New Jersey Medical School, Newark, NJ 07103.

Somatosensory evoked potentials (SEPs), mean blood pressure (MBP) and heart rate (HR) responses were recorded in male Wistar rats anesthetized with urethane, immobilized with tubocurarine and artificially ventilated. Global brain ischemia was induced by the bilateral ligation of vertebral arteries at C1 level followed by bilateral occlusion of common carotid arteries for 3, 6 and 12 min. Control BP and HR were 89.6 ± 12.6 mmHg and 423.1 ± 60.7 bpm, respectively. Control SEPs were characterized by 3 major peaks (P1, P2 and P3) with the onset of 6.8 ± 0.6, 11.0 ± 1.4 and 16.9 ± 1.4 msec, respectively. The amplitudes of these peaks were 0.2 ± 0.1, 2.3 ± 1.7 and 2.0 ± 1.4 uvolts. Global brain ischemia for 3, 6 and 12 min, produced an increase in MBP (42.5, 60.0, and 42.5 mmHg, respectively). Concomitantly there was a decrease in the amplitude of first two peaks (P1 and P2) while P3 disappeared. The changes in BP and SEPs in response to global brain ischemia for 3 min, but not for 6 and 12 min, were reversible. This preparation may serve as a model for global brain ischemia.
Supported by NIH (HL24347) and AHA(NJ).

503.4

PHOTOCHEMICAL NEOCORTICAL STROKE IN RATS: FLUNARIZINE PREVENTS NEUROLOGIC DEFICITS. M. De Ryck, J. Van Reempts", A. Wauquier", M. Borgers" and P.A.J. Janssen". Janssen Research Foundation, B-2340 Beerse, Belgium.

Rose Bengal (20 mg/kg iv) together with topical illumination of the skull causes photo-oxidative injury to cerebral vessels, and focal cerebral necrosis (Watson, B.D., Dietrich, W.D., et al., Ann. Neurol., 17:497, 1985; Van Reempts, J., et al., Stroke, 18:1113, 1987). Does this model of cerebral thrombotic stroke produce enduring neurologic deficits? And, if so, can those be mitigated by posttreatment with the class IV calcium antagonist flunarizine? We made unilateral infarcts in the hindlimb area of the parietal cortex. Flunarizine (1.25 mg/kg iv or 40 mg/kg po) or its solvent was administered 30 min after infarction. We measured neglect, limb placing reactions, and limb usage on elevated beams. On day 21, all rat brains were prepared for histology, including thalamic glial reactivity (typically in ipsilateral nn. VPL and posterior). All control rats displayed sustained deficits in tactile and proprioceptive placing of the contralateral hindlimb. By contrast, placing deficits were dramatically curtailed by flunarizine (FL): 70-75 % of FL rats placed contralaterally on day 1, while the others recovered within 3 to 5 days; but 21-day old infarct volumes and total thalamic density were unaffected. Following cortical infarct, flunarizine preserves behavioral function within a critical period.

503.5

PREVENTION OF MEMORY DEFICIT AFTER ISCHEMIA BY POST-ISCHEMIC INSULIN. C.L. Voll, I.Q. Whishaw and R.N. Auer. Neuroscience Research Group, University of Calgary, Calgary, Alberta, T2N 4N1, and Dept. of Psychology, University of Lethbridge, Lethbridge, Alberta, T1K 4M4.

The ability of post-ischemic insulin to modify the structural and neurobehavioral consequences of cerebral ischemia was studied. Rats were given intraperitoneal glucose 20 minutes before ischemia induced by 10½ min of hypotension and carotid occlusion. Following reperfusion, they were given either insulin (INS) or glucose (GLUC) for one week. Sham operated rats (SHAM) were used as a control group. Rats were trained on a learning-set task 1 - 3 months after ischemia, requiring them to locate an escape platform in a pool of opacified water. Escape latency and swim pattern were recorded. If a rat deviated from an 18 cm wide path to the platform, it received an error on that trial. Performance in the INS group was significantly better in both escape latency and errors ($p<0.05$). Neuropathology predominated in the hippocampus where the GLUC group showed 59% mean neuronal loss compared to 18% mean neuronal loss in the INS group ($p<0.05$). Insulin administration during the early post-ischemic recovery period thus resulted in a significant improvement in performance of behavioral tasks assessing spatial memory and learning ability, in addition to reducing CA1 hippocampal neuronal necrosis.

503.6

AUTONOMIC CHANGES IN RAT FOCAL CEREBRAL ISCHEMIA MODEL. V.C.HACHINSKI, J.X.WILSON*, K.E.SMITH*, D.F.CECHETTO. Robarts Res.Inst./Dept.of Physiol., U. of Western Ontario, London,Ont.N6A5K8.

Acute increases in plasma catecholamines and myocardial damage occur in a cat model of stroke using middle cerebral artery occlusion (MCAO). Similar changes are seen clinically in patients following stroke. Because of the advantages of using a small animal, we examined autonomic changes in two MCAO models of stroke in the rat. Blood pressure (BP), heart rate (HR) and plasma concentrations of norepinephrine (NE) and epinephrine (E) were measured in 20 male, urethane-anesthesized rats that received one of the following treatments: (i) MCAO only, (ii) MCAO and ipsilateral common carotid artery occlusion (MCAO/CCO), (iii) sham occlusions. MCAOs were made immediately distal to the inferior cerebral vein. Arterial blood samples (500 ul) for radioenzymatic assay of NE and E were made twice before the occlusions and at 90 and 180 minutes after the occlusions. At the end of the experiment, tetrazolium salts were reacted with oxidative enzymes to delineate the extent of cerebral ischemia. BP of sham and MCAO/CCO groups significantly declined during the approximately 8 h experiment. However, BP of MCAO rats did not change during the experiment, so that the final BP was significantly higher than in the other two groups. Plasma NE and E concentrations were increased significantly by MCAO compared to pre-occlusion levels and compared to post-occlusion levels in sham and MCAO/CCO groups. These results suggest that focal cerebral ischemia caused by MCAO only in the urethane-anesthesized rat is able to induce the autonomic changes seen in the cat MCAO model of stroke and seen clinically.
(Supported by the Heart and Stroke Foundation of Ontario)

503.7

TEMPORAL PROFILE OF MEMORY AND HIPPOCAMPAL CA1 CHANGES PRODUCED IN 4-VO RAT MODEL OF ISCHEMIA. J.M. Ordy, P. Columbo*, T.T. Volpe, and W.P. Dunlap*, Pennwalt Corp., Rochester, NY 14623, Cornell Med. Center, New York, NY 10021, Tulane Univ., New Orleans, LA 70118.

Patients suffering from cerebral ischemia stroke or cardiac failure have circumscribed memory impairment with selective CA1 hippocampal damage. The four vessel occlusion (4-VO) rat model has been used for examining effects of forebrain ischemia on memory and CA1 cell vulnerability. To differentiate transient from permanent effect CA1 vulnerability, clarification is essential of the temporal progression of memory impairments and degree of viability of CA1 neurons during postischemic periods. The aims of this study were to examine effects of 4-VO ischemia on: 1) spatial working memory and performance from 1 to 4 months post ischemia, 2) topographic distribution and severity of CA1 damage in anterior and mid-dorsal regions of the hippocampus, and 3) correlation of degree of memory impairment with severity of CA1 cell damage. Thirty minutes of 4-VO ischemia significantly reduced working memory without effects on motor performance. Ischemia significantly reduced CA1 neurons in the anterior with lesser damage in mid-dorsal region of the hippocampus. There was a significant correlation between the degree of memory impairment and severity of CA1 cell damage. It remains to be established to what extent this significant correlation was based on compromised but viable neurons during the post-ischemic period.

503.8

CORTICAL FOCAL ISCHEMIA DISRUPTS NON-DISCRIMINATED REVERSAL LEARNING. A. Ortiz*, J.S. MacDonall*, S.P. Mahadik, and S.E. Karpiak. Div. Neuroscience, NYS Psychiatric Inst., Depts. of Psychiatry, and Biochemistry & Molecular Biophysics, Physicians & Surgeons, Columbia U., and Dept. of Psychology, Fordham U., New York.

To determine whether functional deficits after stroke can be improved, we have established a behavioral paradigm sensitive to cortical focal ischemia. Focal ischemia is induced in Sprague-Dawley rats (225-275gr) by permanent unilateral occlusion of the middle cerebral artery & the ipsilateral common carotid artery [MCAo+CCAo] with a 1 hr temporary occlusion of the contralateral CCA. Twelve days after the MCAo rats are trained in a 2-lever chamber to press for food reward. On subsequent days, after 8 consecutive correct responses on the initially reinforced lever, the opposite lever is reinforced. Lever reversals continue after 8 consecutive reinforced responses. The session ends after 10 reversals are completed. Rats with focal ischemia [largely localized to the parietal cortex] show an increase in incorrect responses. But, the largest difference between controls and ischemic rats is an increase in perseverative errors (continuing to press a level even though no reward is provided). We are evaluating the effectiveness of various agents (gangliosides, vitamin E, physostigmine etc.) in reducing this functional deficit, and relating such improvements to changes in edema, Na+, K+, Ca++ in the ischemic areas.

503.9

EFFECTS OF GRADED HYPOVOLEMIC HYPOTENSION ON SPINAL MOTOR EVOKED POTENTIALS IN THE CAT. J.J. Oro*, S.S. Haghighi (SPON: C. Watts) Division of Neurosurgery, University of Missouri, Columbia, MO 65212

Recently, new techniques have been developed to assess motor pathway integrity using electrical or magnetic pulses applied to the cortex. The evoked motor responses have been recorded from the spinal cord, peripheral nerves, or target muscles.

To study the effect of hypotension on spinal motor evoked potentials (SMEPs), we subjected twelve cats to graded hypotension from mean arterial pressure (MAP) of 100 torr down to 30 torr. SMEPs were continuously recorded from spinal cord using epidural surface electrodes. The exposed precruciate motor cortex was stimulated with 150 micro sec, 20 v (max) pulses via AgCl stimulating electrode. The onset latency of SMEPs increased from 3.22 $\pm$ 0.31 msec (n=12) at MAP 100 to 3.6 $\pm$ 0.46 msec (n=10) at MAP$\langle 30 \rangle$40 torr (p 0.05). The peak to peak amplitude was decreased from 7.46 $\pm$ 3.84 μv (n=12) at MAP 100 to 2.96 $\pm$ 2.07 μv (n=10) at MAP$\langle 30 \rangle$40 torr (p 0.05). Conduction velocity decreased from 84.76 $\pm$ 6.81 meter/second (n=12) at MAP 100 to 75.71 $\pm$ 7.81 meter/second (n=10) at MAP$\langle 30 \rangle$40 torr at the rate of approximately 1.2 meter/second/10 degrees of hypotension change. Below 30 torr, 75% of animals lost their SMEPs. Immediate blood transfusion restored SMEPs, however, latency and amplitude did not reach the baseline values within the 1 hour post transfusion period.

503.10

EFFECTS OF HEMORRHAGIC SHOCK ON SPINAL AND CORTICAL SOMATOSENSORY EVOKED POTENTIALS IN THE CAT. S.S. Haghighi, J.J. Oro. Division of Neurosurgery, Univ. Missouri, Columbia, MO 65202.

Somatosensory evoked potentials (SEPs) are being used in the evaluation od spinal and cortical function during surgical procedures. To study the effect of systemic hypotension upon spinal and cortical SEPs, we subjected twelve anesthetized cats to graded hypotension from mean arterial pressure (MAP) of 100 mm Hg down to 30 mm Hg. Spinal and cortical SEPs were recorded to sciatic nerve stimulation. The onset latency of spinal SEPs increased from 1.99$\pm$ 0.47 msec at 100 mm Hg to 2.85$\pm$ 1.04 msec at 30 mm Hg (p=0.05); while conduction velocity (CV) decreased from 95.36$\pm$ 16.22 meter/ second to 71.97$\pm$ 17.05 (p=0.05) at the rate of 2.8 meter/ second/ 10 degrees of hypotension change. Cortical SEPs onset latency increased from 8.42$\pm$0.73 msec at 100 mm Hg to 11.11$\pm$ 1.05 msec at 30 mm Hg (p=0.05). CV decreased with hypotension from 54.76$\pm$ 7.01 meter/ second down to 43.19$\pm$ 6.72 at the rate of 1.5 meter/ second/ 10 degrees hypotension. No cortical SEPs were detectable below 30 mm Hg. Spinal evoked responses were more resistant to profound hypotension and disappeared last. Blood transfusion resumed spinal SEPs first followed by cortical SEPs.

These findings suggest that ischemia associated with profound systemic hypotension can alter evoked responses.

503.11

NEW INHIBITORS OF LIPID PEROXIDATION. M.Gall*, R.I.Higuchi*, E.D.Hall, and J.M.Braughler (SPON: B.D.Greenberg), CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Amino-substituted 9,10-secosteroids are potent inhibitors of iron-dependent lipid peroxidation as determined in the malondialdehyde (IC_{50} values between 18 and 80 μM) and conjugated diene assays. In addition, they protect animals from the sequelae of head injury damage. In collaboration with Prof. T.Kuwana and associates (CBAR, Univ. of Kansas), we report redox potential data which suggests that the amino heterocycle structure of the secosteroids, and their steroidal parents (ie, Lazaroids,) may serve as a radical-quenching reducing-agent in biological systems.

503.12

INCREASE IN EXTRACELLULAR ASCORBATE DURING FOCAL CEREBRAL ISCHEMIA IN THE RAT MONITORED BY INTRACEREBRAL MICRODIALYSIS. L.Hillered (1,2), L.Persson*(2) and U.Ungerstedt*(3). Depts. of Clinical Chemistry(1) and Neurosurgery(2), University Hospital, S-751 85 Uppsala, and Dept. of Pharmacology(3), Karolinska Institute, Stockholm, Sweden.

Apart from its role as a major antioxidant in the brain, ascorbate (AA) is well known to have the paradoxical ability to induce iron-dependent lipid peroxidation. Recently, AA has been implicated as an important modulator of neostriatal activity. Administration of AA (i.p.) appears to increase neuronal activity in the striatum. It was therefor of interest to measure the extracellular levels of ascrobate during focal cerebral ischemia. Microdialysis probes (Carnegie Medicin AB, Stockholm, Sweden; membrane lenght 3 mm) were implanted stereotactically into the caudato-putamen bilaterally. Dialysis was started 2 hours later using Ringer solution at a flow rate of 2 ul/min. Samples were collected in three 30-min fractions before and six after the onset of ischemia. Ischemia was induced by middle cerebral artery occlusion on the left side. Compared to the pre-occlusion level and to the contralateral side ischemia was associated with a 4-6 fold increase in ascorbate in dialysates from the left striatum. The level of ascorbate did not change significantly on the contralateral side. We propose that ascorbate may aggravate neuronal injury in the ischemic penumbra by the induction of lipid peroxidation and/or by an excitotoxic mechanism.

503.13

CONTINUOUS TRANSCRANIAL MONITORING BY LASER DOPPLER VELOCIMETRY OF CEREBRAL BLOOD FLOW CHANGES FOLLOWING TRANSIENT ISCHEMIAS. S. Xu*, H.G. Wagner, F. Joo*, and I. Klatzo. Lab. of Neuropathol. & Neuroanat. Sci., Nat. Institutes of Health, Bethesda, MD 20892

Dynamic changes of cerebral blood flow (CBF) induced by multiple transient bilateral carotid occlusions were studied in the Mongolian gerbil. The data obtained by transcranial measurement of CBF by Laser Doppler Velocimetry (LDV) correlate well with that obtained by intracranial LDV. LDV technique made possible repeated and continuous measurement of these circulatory parameters over long periods of time with minimal attention to the hazards of exposure or infection of the brain. Transcranial LDV provided immediate information about the preischemic normalcy, the completeness of the ischemia and the recovery of blood flow following release of the occlusion which in our studies was a function of the duration of the ischemias. In very short term ischemias, LDV showed that recovery of blood flow was immediate. With longer ischemias CBF recovered partially, followed by slower further increase and formed a hyperemic peak, often with some overshoot. The CBF then slowly fell to a hypoperfusion stage. The effects of certain vasoactive drugs on this sequence were studied by LDV and demonstrated the usefulness of LDV to evaluate these substances.

503.14

A NEW MODEL TO STUDY THE ACTIONS OF REACTIVE OXYGEN SPECIES ON MAMMALIAN NEURAL TISSUE. L. Roskos*, R.K. Evans*, V. Centenera* and F.C. Kauffman. Dept. of Pharmacol. and Exper. Therap., Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Hippocampal slices from adult rat brain were used to study biochemical mechanisms of injury to neural tissue due to reactive oxygen species. The hippocampus was selected as a model because this area of brain is exceptionally sensitive to ischemia and is used widely in neurochemical and electrophysiological studies. Methylene blue (MB), a compound known to undergo redox cycling, and H_2O_2 were used to injure the tissue. Hippocampal slices could be maintained under nitrogen for at least 15 minutes with no change in energy status after recovery under 95% O_2:5% CO_2 for 3 h. The effects of reactive O_2 species and protective agents were examined in slices recovering from anoxia and in tissues that had fully recovered. Redox cycling of MB and generation of H_2O_2 occurred via a photosensitive reaction. Thus, experiments with MB were carried out in light and dark. MB produced decreases in ATP and P-creatine in the light but not in the dark. Protective agents tested include: desferoxamine, an iron chelating agent; verapamil, a Ca^{++} channel blocking agent; allopurinol, an inhibitor of xanthine oxidase; and catechin, a free radical savenger. These agents did not protect slices recovering from anoxia but did provide some protection against reactive O_2 species in slices that had recovered. All agents inhibited recovery of energy status in tissues incubated with MB in the dark suggesting that cellular injury due to MB involves events in addition to formation of reactive oxygen species. The results argue strongly that different pharmacological strategies are required to combat the actions of damaging O_2 species on neural tissue recovering from anoxia than those used to alter the actions of reactive O_2 species on normal neural tissue. (Supported in part by NIH Grant HD-16596).

503.15

PROTECTIVE EFFICACY OF THE LIPID PEROXIDATION INHIBITOR U74006F ON POST-ISCHEMIC CA_1 DEGENERATION IN GERBILS AFTER BRIEF BILATERAL VS. PROLONGED UNILATERAL CAROTID OCCLUSION: EVIDENCE FOR DIFFERENT DEGENERATIVE MECHANISMS. K.E. Pazara* and E.D. Hall (SPON: J.M. Braughler). CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

A comparison was made of the efficacy of the 21-aminosteroid oxygen radical scavenger and lipid peroxidation inhibitor U74006F to antagonize post-ischemic degeneration in the hippocampal CA_1 region of mal Mongolian gerbils after either a 10 min. bilateral occlusion (BCO) of the carotid arteries or a 3 hr. occlusion of one carotid artery (UCO). At 1 week after the 10 min. BCO, pronounced (-75.4%), but selective CA_1 cell loss was apparent. Pretreatment (30 min.) with U74006F over a wide range of i.p. doses (1, 10 or 30 mg/kg) plus a second dose at 2 hrs. after BCO had no effect on the CA_1 cell loss. In the 3 hr. UCO model, a similar degree of CA_1 cell loss was observed after only 24 hrs. in addition to diffuse degeneration throughout the ipsilateral hemisphere. In this model, i.p. pretreatment with U74006F (10 mg/kg) plus a second dose at the end of the 3 hr. UCO significantly reduced CA_1 damage as well as the cell loss in other brain areas (e.g. cortex). These results suggest that the mechanism of CA_1 degeneration may differ between ischemia models with oxygen radical-induced lipid peroxidation perhaps being more involved in the 3 hr. UCO model.

COMPARATIVE NEUROANATOMY: CEREBRAL CORTEX

504.1

A HYPERCARD GLOSSARY OF MACAQUE AND HUMAN NEURO-ANATOMICAL NOMENCLATURE. D.M. Bowden and R.F. Martin. Dept. Psychiatry and Beh. Sci. and Regional Primate Research Center SJ-50, University of Washington, Seattle, WA 98195

The Macaque/Human-Glossary of Neuroanatomical Nomenclature allows the user to identify the brain structure to which any generally accepted English or Latin neuroanatomical name applies. When the user enters a structure name into the computer, the computer responds with a list of all accepted synonyms and with references to one or more macaque and human brain atlases in which the structure is defined or illustrated. The computer shows where in the hierarchy of structures and substructures the named structure lies, i.e., button clicks take the user up the limb of suprastructures or out the branches of substructures to indicate how the named structure relates to other parts of the brain. The glossary includes a total of 750 structure names and more than 2300 synonyms. The hierarchy is based on the human Nomina Anatomica with extensions based on several macaque and human brain atlases.

Supported by NIH grant RR00166 to the University of Washington.

504.2

ENCEPHALIZATION QUOTIENT AND REGIONAL BRAIN MORPHOMETRY IN THE THE WEST INDIAN MANATEE. R.L. Reep and T.J. O'Shea*. Dept. Neurosci., J-244, Univ. Florida; and USFWS, 410 NE 16th Ave.; Gainesville, FL 32610 and 32601.

We determined an encephalization quotient (EQ) of 0.275 for T. manatus, based on direct measurements of brain mass (avg-364g) and body mass (avg-756kg) in 13 specimens. This is among the lowest values for all mammals and is comparable to EQ estimates for other Sirenia species. However, when our data are adjusted for metabolic rate, actual brain weight is 1.5 times larger than predicted. The unique aquatic herbivorous lifestyle of the Sirenia has likely been a major determinant of large body size and low metabolic rate. We suggest that early in Sirenia history brain-body size allometry was uncoupled, permitting selection for an increase in body size without a corresponding change in relative brain size. Low metabolic rate may also be a factor in constraining brain size in manatees.

Gyration of the cerebral cortex, usually positively correlated with absolute brain size, is strikingly absent in these brains. However, morphometric data indicate that the telencephalon comprises 71% of total brain volume in T. manatus. This is comparable to values for prosimians and monkeys and much larger than values for insectivores and bats. Likewise, manatee cerebral cortex is well laminated and of robust cellular density. A retarded growth curve for the brain may explain the lissencephalic condition. In any case, internal structural complexity of the brain appears to have been unaffected by the size restrictions implied by low EQ, or by the lissencephaly.

Supported by grant BSR-03687 from NSF, and cooperation of US Fish and Wildlife Service, Florida DNR, and Sea World of Florida, Inc.

504.3

NEURONAL ORGANIZATION AND MICROCIRCUITRY OF LAYERS I AND II IN VISUAL CORTEX OF DOLPHINS (STENELLA COERULEOALBA AND TURSIOPS TRUNCATUS). P.J. Morgane and I.I. Glezer. Worcester Foundation for Experimental Biology., Shrewsbury, MA 01545, and CUNY Medical School., New York, NY 10031.

The neuronal composition and microcircuitry of layers I and II of the dolphin visual cortex were analyzed. We have found that these cortical layers are generally similar to those seen in most conservative eutherian mammals such as basal Insectivora. Layer I is most predominant and occupies 1/3 of the entire cortical width. Layer II is characterized by an extremely dense concentration of neurons. By using rapid Golgi impregnation, we were able to analyze the neuronal spectrum of layer II as well as the axonal and dendritic composition of layer I in the dolphin neocortex. The main neuronal types seen in layer II are transitional pyramids with slender apical dendrites extending widely into layer I and, secondarily, other transitional pyramids with two apical dendrites which ascend widely into layer I ("extraverted" neurons). The neuronal organization of layers I and II in the dolphin neocortex provides the preliminary basis for our hypothesis stating that these layers are the main afferent input layers from subcortex and for associative fibers, providing intracortical connections. (Supp. by NSF grants BNS 85-45732, 87-42032, CUNY grants BRS 442-2490, 6-67-204 and the Osborn Laboratories of the New York Aquarium of New York Zoological Society).

504.4

ULTRASTRUCTURE OF SYNAPSES IN VISUAL CORTEX OF THE DOLPHIN (STENELLA COERULEOALBA). I.I. Glezer and P.J. Morgane. CUNY Medical School., New York, NY and Worcester Foundation for Experimental Biology., Shrewsbury, MA 01545.

Qualitative and computerized quantitative analyses of ultrastructural features of synapses in different layers of visual cortex in the dolphin were carried out. Blocks from the area representing the primary visual projection zone were processed for transmission electron microscopy and rapid Golgi technique. Several features characterize the visual neocortex of the dolphin: the density of synapses in dolphin visual cortex is significantly higher than in the visual cortices of most terrestrial mammals and is comparable to that seen in some rodents (rats and mice). Another feature is the presence of extremely large numbers of synapses en passage which are especially abundant in layer I. Multiple vesicle types are seen in nearly all synaptic boutons. Most of the ascending collaterals of subcortical and intracortical axons reach layer I in the dolphin convexity neocortex. The sum of the volume of synaptic boutons per cu. mm was significantly higher in layer I than in other layers. Overall, several features of synaptic organization support our general concept of the special afferent role of layer I which may be an important level of cortical integration in the whale brain. (Supported by NSF grants BNS 85-45732, 87-42032, CUNY grants BRS 442-2490, 6-67-204 and the Osborn Laboratories of the New York Aquarium of New York Zoological Society).

504.5

THE DEGREE OF CORTICAL FOLDING IN PRIMATE BRAINS. K. Zilles,[1] E. Armstrong,[2,3] and A. Schleicher.[1] Anatomisches Inst., Univ. Koln, Koln, F.R.G.,[1] Yakovlev Collection, A.F.I.P., Washington, D.C.[2] and Dept. Anatomy, U.S.U.H.S., Bethesda, MD.[3]

The degree of cortical folding in primates varies from lissencephalic prosimians to the highly convoluted human brain and can be analyzed in serial coronal sections by measuring the lengths of the complete outer cortical contours and those parts which are superficially exposed. Ratios of the two lengths, the gyrification index (GI), were collected for 42 different primate species. GI was studied as a function of brain and body weights, and for differences in lateralization.

The mean GI of the human brain (N = 61) fits well within the anthropoid correlations of GI and brain and body weights. Anthropoid GI's are highest in the posterior parietal regions and taper rostrally with bigger brains tapering more gradually. The larger human GI is the result of a more convoluted frontal lobe. While bigger primate brains are more convoluted, for every unit increase in brain size prosimian brains gain fewer convolutions than do anthropoid brains. Within Homo GI is not associated with differences in brain size nor were differences between right and left hemispheres observed. GI symmetry in the temporal plane suggests that the observed surface lateralization here comes from an absolute increase in cortex, not in its folding.

504.6

THE ONTOGENY OF CORTICAL FOLDING IN THE HUMAN BRAIN. E. Armstrong[1,2], K. Zilles[2], M. Pan[1,2*] and A. Schleicher[2*]. Yakovlev Collection, A.F.I.P., Washington, D.C.[1], Dept. Anatomy, U.S.U.H.S., Bethesda, MD.[2], and Anatomishes Inst., Univ. Koln, Koln, F.R.G.[2].

Cortical folding in serially sectioned human brains was analyzed by measuring the lengths of the total cortical contour and dividing that by the lengths of the superficially exposed parts of this contour. This ratio or gyrification index (GI) was determined in 26 human brains ranging in age from 11 to 400 weeks postconception. The cerebral cortex is lissencephalic (GI <1.1) until week 25. The adult degree of folding is reached around 2.5 months postnatally. The increase in GI occurs when the brain grows from 100-600 g. Although the human brain doubles in size after that, the GI remains stable. The degree of convolutedness is thought to result from a more predominant growth in the outer than in the inner cortical laminae. The temporal pattern of GI development revealed in our study suggests that the degree of gyrification is not correlated with number of neurons (most of the cortical neurons have migrated to the cortical plate before GI increases), but more with the development of cell processes and interconnections which is on-going during this time. The myelination of cortico-cortical tracts begins after GI has stabilized suggesting that the space occupied by the former does not play a critical role in gyrification.

BEHAVIORAL DISORDERS

505.1

CANNABINOID POTENTIATION OF NEUROLEPTIC-INDUCED HYPOKINESIA: THE NICOTINIC HYPOTHESIS OF ACTION. D.E. Moss, P.Z. Manderscheid, A.B. Norman, and P.R. Sanberg. University of Texas at El Paso, El Paso, Texas 79968 and University of Cincinnati School of Medicine, Cincinnati, Ohio 45267

Interest in cannabinoids and nicotine in the treatment of motor disorders resulted from the discovery that cannabinoids or nicotine (NIC) potentiate neuroleptic-induced hypokinesia up to 100 fold. It has been proposed that cannabinoids and NIC produce this powerful effect through a CNS nicotinic cholinergic mechanism. The purpose of these experiments was, therefore, to determine if mecamylamine (MEC, a nicotinic antagonist) would reduce the effects of NIC and cannabinoids.

Rats were pretreated with fluphenazine HCl (FLU, 0.1 mg/kg IP) and hypokinesia was measured by the bar test. Some rats received an injection of MEC (1.0 mg/kg IP) 1 hr after FLU. MEC pretreatment significantly reduced the subsequent effect of NIC (0.1 mg/kg IP) or delta-9-THC (10 mg/kg gavage).

These results support the hypothesis that cannabinoids act through a nicotinic mechanism and further support the hypothesis that certain cannabinoids may be useful to increase the efficacy of neuroleptics in motor disorders.

505.2

NICOTINE MARKEDLY POTENTIATES NEUROLEPTIC-INDUCED CATALEPSY. P.Z. Manderscheid, P.R. Sanberg, A.B. Norman, H.M. Fogelson*, B.J. McConville*, and D.E. Moss. Lab. of Behavioral Neuroscience, Depts. of Psychology and Psychiatry, Univ. of Cincinnati College of Med., Cincinnati, OH 45267.

Haloperidol, as well as other neuroleptics, is widely used in treating hyperkinetic movement disorders. Recently, we found that nicotine produced a 10-fold increase in reserpine-induced catalepsy. Since catalepsy may provide a useful model of extrapyramidal function, the present study investigated the effect of nicotine on haloperidol-induced catalepsy in an effort to elucidate the role of nicotine in modulating the function of the extrapyramidal system.

Male Sprague-Dawley rats, age 3 to 6 months were randomly assigned to one of four treatment groups: haloperidol plus nicotine, haloperidol only, nicotine only, and vehicle control (n=8/group). Haloperidol was given in doses of 0.1, 0.3, or 0.5 mg/kg prior to nicotine (0.1 mg/kg). The bar test was used to measure catalepsy. Nicotine produced an almost 10-fold increase in catalepsy in the 0.3 mg/kg haloperidol group ($F(1,14)=9.84$, $p<.01$). At 0.1 mg/kg haloperidol, nicotine produced only a mild potentiation of catalepsy and, at 0.5 mg/kg haloperidol, the interaction was not apparent due to a ceiling effect produced by haloperidol.

This animal model may be important in elucidating the mechanisms of interaction between neuroleptics and nicotine. Insofar as neuroleptics are used to treat movement disorders such as Tourette's Syndrome and Huntington's disease, nicotine may be considered as an adjunct treatment which potentiates the therapeutic efficacy of neuroleptics.

505.3

AUTOTOMY INDUCED BY SUBCUTANEOUS INJECTION OF HYPERTONIC SALINE IN THE TAIL OF THE RAT. T.I. Kanui* and G.M.O. Maloiy* (SPON: K. Hole), Dept. of Animal Physiol., Univ. of Nairobi, P.O. Box 30197, Nairobi, Kenya.

The basis of autotomy behaviour still remains unknown (Rabin, A.G. et al., Pain, 21:117-128). The phenomenon was studied in 30 Wistar rats (150-450g). A standard dose of 0.125 ml 10% saline injected subcutaneously, 2 cm from the tip of the tail was used.

For the first 2 h, the rats licked the site of injection and also proximal to this. Three categories of responses were recognised. Fourteen rats neither showed autotomy or licking behaviour. Six rats showed autotomy (bites, blood stain) that resolved in 2.26 ± 1.52 (mean ± S.D.) weeks without signs of ischaemia and subsequent amputation. Ten rats licked, bit and showed signs of ischaemia, followed by amputation of the tail in 8 ± 3.3 weeks. The onset was 2.26 ± 1.52 weeks.

A second application of 10% saline in animals showing autotomy, resulted in autotomy in 40% of the cases. After autotomy, no signs of phantom limb pain were seen for a period of 1 year.

The data presented suggest that peripheral chronic pain may initiate autotomy followed by signs of ischaemia and necrosis which may lead to amputation.

Rabin, A.G. et al., Pain, 21:117-128, 1985.

505.4

NICOTINE GUM AND HALOPERIDOL IMPROVE TOURETTE'S SYNDROME. P.R. Sanberg, H.M. Fogelson,* P.Z. Manderscheid, K.W. Parker,* A.B. Norman, K.J. Hart,* F.P. Zemlan, and B.J. McConville,* Lab. of Behavioral Neuroscience, Dept. of Psychiatry, College of Medicine, and Dept. of Ped. Neurology, Children's Hospital, University of Cincinnati, Cincinnati, OH 45267.

Tourette's Syndrome is a complex disorder with both motor and verbal tics. While the drugs of choice for this disorder are dopamine receptor blockers, such as haloperidol, some patients show only marginal response. Furthermore, these drugs can lead to sedation, exacerbation of learning difficulties, and possible tardive dyskinesia. For these reasons, an agent that potentiates the effects of neuroleptics could allow for lower doses of neuroleptics and reduction in side effects.

In animals it has been shown that nicotine markedly potentiates the behavioral effects of haloperidol (Moss et al., and Manderscheid et al.; this issue). Nicotine gum was given to patients with Tourette's Syndrome who showed only moderate responses to haloperidol alone. A marked reduction in the intensity of tics and an increase in attention and concentration was seen in six of the seven cases. In some cases the tics stopped completely. This effect started after 15 minutes, lasting about one hour. Side effects including bitter taste and gastric distress often caused non-compliance. Chewing regular gum did not effect tics. Two patients with Tourette's Syndrome given nicotine without haloperidol showed no such beneficial effects. Although the mechanism for this beneficial effect of nicotine needs to be elucidated, nicotine may prove useful in treating neuroleptic-responsive disorders.

505.5

DECREASED IL-2 PRODUCTION, INCREASED IL-2 RECEPTORS, AND REDUCED CD8 LYMPHOCYTES IN SCHIZOPHRENIA. B.S. Rabin*, R. Ganguli*, (Spon: J. Huff). Dept. Psychiatry, Univ. Pittsburgh Med. School, Pittsburgh, PA 15213.

We have previously reported that schizophrenics show evidence of multiple autoantibodies, circulating antibody to normal brain tissue and increased mitogenic responses to brain antigens. These data can be interpreted as suggesting a disorder with an autoimmune pathogenesis. To further investigate this possibility we studied other immune parameters which are reported to be abnormal in autoimmune disease. 70 RDC schizophrenic patients had IL-2 production levels measured in morning fasting blood samples. 50 non-psychiatric controls were also studied. Analysis of variance showed that patients produced significantly less IL-2 than controls. Acutely ill patients, whether medicated or not produced significantly less IL-2 than either remitted patients or controls. Age, race and sex were not found to be correlated with IL-2 production. CD8+ and CD4+ cells were quantitated in all subjects. Regardless of whether they were medicated or not, CD8 cells and CD4+2H4+ (the inducer of suppressor cells) were significantly lower in the schizophrenic patients. Soluble interleukin-2 receptor, which is increased in autoimmune disease, was significantly increased in patients with schizophrenia. These data while not proving an autoimmune pathogenesis in schizophrenia, do support that possibility.

505.6

PERINATAL BRAIN INJURY VS GENETIC LOADING IN SCHIZOPHRENIA: MAGNETIC RESONANCE IMAGING FINDINGS. H.A. NASRALLAH, S.C. OLSON*, J.A. COFFMAN AND S.B. SCHWARZKOPF*. Department of Psychiatry and The Neuroscience Program, Ohio State University College of Medicine, Columbus, OH 43210.

Several lines of evidence suggest that genetic factors are important in schizophrenia. A substantial literature also points to an increased frequency of perinatal brain injury, some of which (in the second trimester) can impair cell elimination or migration. Cortical atrophic changes have been described in young schizophrenic patients. We conducted a Magnetic Resonance Imaging (MRI) study to compare the effects of genetic loading and perinatal complications on brain structure in schizophrenia, with the hypothesis that atrophy is more likely to be associated with perinatal brain injury than with genetic loading.

Forty schizophrenic patients (by DSM III-R) were interviewed with their parents for first degree family history (FH+) of psychosis and for various perinatal brain injuries. MRI scans were obtained (TR=1500 MS, TI=800 MS). Midsagittal cerebral, cranial, frontal and ventricular areas were measured. No significant correlations were found between perinatal events and MRI variables. On the other hand, FH+ correlated significantly with smaller cranial size (p .04) and with a strong trend for smaller cerebral and frontal size as well. The results indicate that developmental factors (such as a small cranium, not just a small cerebrum) may be associated with genetic rather than perinatal factors.

505.7

QUANTITATIVE EVALUATION OF IODOAMPHETAMINE SPECT STUDIES IN PARANOID SCHIZOPHRENICS. D.S. Schlusselberg, W.K. Smith, T.R. Simon*, J.D. Raese, and D.J. Woodward, *UT Southwestern Medical Center, Dallas VA Medical Center, Dallas, Texas.*

Ongoing studies have been performed to evaluate the cerebral uptake of I-123-Iodoamphetamine (IMP) with Single Photon Emission Computed Tomography (SPECT). Because of differences in brain size, shape, and position, three-dimensional (3D) alignment is necessary to quantitatively compare different studies. Algorithms have been developed for computer processing of 3D volumes formed by a set of serial slices, that include 3D translation, rotation, and scaling. The volume of data is then resampled at a lower resolution to provide a spatially normalized data set for statistical analysis. Voxel counts are also normalized to account for differences in the amount of IMP injected and variable uptake in non-cerebral tissues.

Studies from 18 normals were processed to calculate the mean and standard deviation for each resampled voxel. 22 DSM-III classified chronic paranoid schizophrenic patients (CPS) were evaluated for regional statistical differences from the normal group. Quantitative differences between frontal and temporal activity in normals versus CPS were confirmed. This method offers an objective quantifiable evaluation of regional IMP uptake. *(Support from the Biological Humanics Foundation and NIDA 2938)*

505.8

QUANTITATION OF ASTROCYTES IN THE MOLECULAR LAYER OF THE DENTATE GYRUS: A STUDY IN SCHIZOPHRENIC AND ALZHEIMER'S DISEASE PATIENTS. M. F. Casanova*, J. Stevens and J. E. Kleinman. Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032.

Recently several authors have claimed prominent cytoarchitectural abnormalities in the entorhinal cortex of both Alzheimer's disease (AD) and schizophrenic patients (SC). In the present study, we have attempted to corroborate the presence of such a lesion in both AD and SC patients by quantitating astrocytic markers within the terminal fields of the perforant pathway. Hippocampal blocks from 6 SC, 4 AD and 7 neurologically impaired controls were taken anterior to the lateral geniculate nucleus. The sections were stained with the Holzer technique and analyzed with a LOATS computerized morphometry system. Our results indicate marked abnormalities only for the AD patients. Since the redistribution and hyperplasia of astrocytes within the molecular layer of the deafferented gyrus depends on the chronicity of the entorhinal lesion, the abnormalities observed in AD patients suggests that the underlying etiology pursues a protracted course. The absence of changes in SC patients does not disprove entorhinal pathology but rather suggests that if a lesion exists, it is either static in nature or occurred long before the postmortem examination of the tissue sample.

505.9

BRAIN DENSITY IN SCHIZOPHRENIA. D. Kostianovsky*, E. Kim*, D. G. Daniel*, T. Goldberg*, M. Casanova, J. E. Kleinman and D. R. Weinberger (SPON: M. E. Dorman). Clinical Brain Disorers Branch, NIMH, Washington, D.C. 20032.

Several previous studies have demonstrated differences in regional brain density (as derived from CT scan attenuation values) between patients with schizophrenia and normal controls. Interpretation of these studies has been hindered by methodological shortcomings such as failure to control for head size, scanner calibration differences and other confounding variables. The present study offered methodological advances over earlier studies by controlling for head size and normalizing the attenuation values for each scan to an internal standard. CT attenuation values in multiple brain regions in 20 patients with chronic schizophrenia were compared with those of 20 age and sex matched controls. No significant differences in regional attenuation values emerged between the schizophrenics and normal controls. The results confirm the importance of controlling for artifacts in analysis of CT scan attenuation values and raise questions about the validity of regional CT attenuation values in detecting subtle anatomical abnormalities in schizophrenia.

505.10

BRAIN METABOLISM DURING AUDITORY HALLUCINATIONS. J.M. Cleghorn. Dept. of Psychiatry, McMaster University, Hamilton, Ontario Canada L8N 3Z5.

These studies attempt to locate regions of the brain associated with auditory hallucinations by means of positron emission tomography with [^{18}F] fluoro-deoxy-glucose. Data on two samples will be reported. I: first episode drug naive psychotic patients (N=17) of whom 11 were hallucinating and 6 were not hallucinating during the glucose uptake period prior to the scan. II: chronic patients (N=19) medicated for an averge of 7.4 years, in whom auditory hallucinations were still present in 9 and had disappeared in 10.

Hallucinating (H) and nonhallucinating (NonH) groups did not differ from each other in the regions in which glucose metabolism was measured: prefrontal and orbito-frontal cortex, parietal cortex, superior temporal and Wernicke's cortex. Broca's and auditory cortex metabolism was also similar in H and NonH groups.

However, correlations between some brain regions characterized both H groups and were not observed in the NonH groups: right hemisphere regions homologous for Broca's and auditory areas $r = >+.81$ ($p<.01$) and right frontal and parietal $r = >+.80$ ($p<.01$). In the drug free H group the right sided homologous regions for Broca's and Wernicke's areas $r = +.89$ $p < .001$.

These studies suggest that right hemisphere regions homologous to language areas on the left are coupled during auditory hallucinations.

505.11

NON-STATE DEPENDENT CHANGES IN PLATELET 3H-IMIPRAMINE BINDING WITH CHRONIC IMIPRAMINE TREATMENT. K.M. Bell, E.M. DeMet, R. Gerner*, C. Kauffmann*, A. Chicz-DeMet. Dept. Psychiatry, Univ. of Calif., Irvine, CA. 92717.

Unipolar depressed patients were treated with imipramine (IMI) for 4-13 wks. Bmax values were increased by 33% after 4 wks but later returned to baseline even though the patients remained euthymic. These changes were independent of plasma drug levels and were not due to drug carryover since the latter was demonstrated to alter the Kd with no change in Bmax *in vitro*. Post- treatment Bmax and plasma cortisol were inversely related but were uncorrelated at baseline and there was no difference between pre- and post-treatment cortisol levels. Treatment response was not significantly correlated with either the Bmax or plasma cortisol changes, although relatively poor responses were associated with extreme values (high and low) of pretreatment Bmax. The results suggest that chronic IMI treatment results in transient increases in Bmax through a cortisol dependent mechanism. Since these changes were not related to therapeutic response, the results further suggest the limited usefulness of 4-13 week drug trials in the assessment of the state dependence of platelet binding.

505.12

DEPRESSIVE DISORDERS FOLLOWING POSTERIOR CIRCULATION AS COMPARED WITH MIDDLE CEREBRAL ARTERY INFARCTS. S.E. Starkstein* and R.G. Robinson (SPON: P.R. McHugh) Dept. of Psychiatry, Johns Hopkins Univ Sch Med (Baltimore, MD 21205)

Patients with cerebrovascular lesions in the posterior circulation (PC) territory (n=37) were compared with patients having middle cerebral artery (MCA) (n=42) strokes for the presence of mood disorders. While both groups showed a similar profile of clinical symptoms of depression during the acute evaluation in-hospital, patients with PC lesions involving the brainstem and/or cerebellum demonstrated a significantly lower frequency of depression (27%) than patients with MCA lesions (48%) or patients with PC lesions involving the left cerebral hemisphere (100%). Moreover, at two-year follow-up, depression following brainstem and/or cerebellar infarcts was significantly shorter in duration than depression following MCA lesions (mean Present State Exam depression scores from in-hospital to 2 years follow-up were significantly lower for the brainstem/cerebellar group than the MCA patients with in-hospital depression over the same period, repeated measures ANOVA group by time $F=6.14$, $df=1,23$, $p<.05$). These differences in the frequency and duration of depression following brainstem/cerebellar as compared with MCA lesions were not explained by differences in lesion volume, physical impairment, cognitive deficits or quality of social support. They suggest that PC and MCA-induced depression may have different aetiologies.

505.13

Responses to clonidine in acute and remitted depressed patients.

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Clonidine, an alpha$_2$-adrenergic agonist which results in increases in plasma growth hormone (GH) and decreases in plasma 3-methoxy-4-hydroxyphenylglycol (MHPG), was administered to 28 acute depressed patients, 15 remitted depressed patients, and 12 normal controls. All subjects were free of medical illness, drug-free for at least two weeks, and on a low monoamine diet for three days prior to testing. A reduced growth hormone response to clonidine (< 5 ng/ml) was found in 68% (19/28) of the acute depressed patients and in 67% (10/15) of the remitted depressed patients as compared to 25% (3/12) of the age-and sex-matched controls (acute normal, remitted normal, Fisher's Exact, $p< 0.05$). All patients initially blunted in the acute state remained blunted in the remitted state. Acute depressed patients demonstrated lower absolute (0.1 ± 0.5) or percent decrease ($0.9 \pm 10.7\%$) in plasma MHPG compared to normal controls (absolute: 0.4 ± 0.6, percent: 8.4 ± 14.8) (t-test, $p<0.05$), while remitted patients were similar to controls. These results suggest that the GH response to clonidine is blunted in depressed patients regardless of state, while the reduced MHPG response to clonidine is state-dependent.

505.14

SECONDARY vs IDIOPATHIC MANIA B. Boffi*, R. C. Young, G. Klerman* (SPON: M. Russ). The New York Hospital-Cornell Medical Center, Westchester Division, White Plains, NY 10605.

"Secondary" or symptomatic manic syndromes - those presenting in patients with associated medical disorders or drugs implicated as etiologic - have not been studied systematically. We retrospectively studied patients admitted over a three year period to two university psychiatric hospitals. Patients with a DSM III diagnosis of organic affective disorder, manic (N = 12) and with bipolar disorder, manic (N = 212) were contrasted using a computerized data base. Organic patients were older at index hospitalization (mean age 49.3 yrs $\pm$ 34.4 yrs, S.D., vs. 38.3 yrs $\pm$ 16.4 yrs; $p< .14$), were older at first psychiatric hospitalization (45.8 yrs $\pm$ 22.8 yrs vs. 31.0 yrs $\pm$ 15.1 yrs; $p< .01$), and had fewer hospitalizations (1.3 ± 0.9 vs 3.0 ± 2.8; $p< .001$). They had longer duration of index hospitalization (54.6 days $\pm$ 29.5 days vs. 37.0 days $\pm$ 34.8 days; $p< .07$), lower Global Assessment Scale (GAS) scores at discharge (41.3 ± 9.3 vs. 56.0 ± 13.8; $p< .001$), and less improvement in GAS scores during hospitalization (10.5 ± 10.4 vs. 22.5 ± 16.7; $p< .01$). These preliminary findings suggest that "secondary mania" is a syndrome with later age at onset and poorer treatment outcome.

505.15

ANTIDEPRESSANTS AND LATE LIFE MANIA. H. Jain* and R. C. Young. The New York Hospital-Cornell Medical Center, Westchester Division, White Plains, New York 10605.

Manic episodes can occurr for the first time in late life. In such patients, etiologic factors and pathophysiologies may differ from those in patients in whom mania occurs first in early life. Charts of geriatric (age ≥ 60 yrs.) psychiatric inpatients (N=40) who met DSM III criteria for bipolar disorder, manic, were reviewed. The median age at occurrence of first manic episode was 58 years, and the range was 18 to 81 yrs. Seven (35%) of the patients with later age at occurrence of first manic episode had been treated with antidepressant drugs in association with development of the index manic episode, compared to one (5%) of the patients with earlier age at occurrence of first manic episode (Fisher exact test, p=.027). Six patients had been treated with tricyclic antidepressants, one with maprotyline, and one with phenelzine. Later and earlier onset patients did not differ in number of treated depressive episodes. The relationship between antidepressant pharmacotherapy and illness course in bipolar disorder remains controversial. These preliminary findings suggest that geriatric bipolar patients with later age at occurrence of first manic episode are more vulnerable to induction of mania by antidepressant drugs than are geriatric patients with earlier first occurrence of mania.

505.16

MOOD CHANGES FOLLOWING RIGHT HEMISPHERE LESIONS. M.A.Honig* S.E.Starkstein* and R.G.Robinson (SPON: J.B.Wirth). Dept. of Psychiatry, Johns Hopkins Univ Sch Med, Baltimore, MD 21205

Ninety-three patients with right hemisphere (RH) ischemic or hemorrhagic stroke lesions were examined for mood changes. Within 2 months after stroke, 49% showed no mood changes, 18% developed major depression, 12% developed minor depression and 21% showed an unduly cheerful mood. Patients with depression (both major or minor) had a significantly higher frequency of lesions involving the parietal cortex as compared to non-depressed or unduly cheerful patients (X^2=9.6, p=.022). In addition, patients with major depression had a significantly higher frequency of family history of psychiatric disorders than: 1) patients with RH lesions and no mood disorders (X^2=4.08, p<.05); and 2) another group of 27 patients with major depression following left hemisphere lesions (X^2=4.44, p<.05). Patients displaying undue cheerfulness showed a significantly higher frequency of lesions involving the frontal operculum than depressed and non-depressed patients (X^2=7.776, p=.05).

In conclusion, depression after right hemisphere lesions are associated with parietal damage. Genetic factors also seem to play an important role in major depression following right but not left hemisphere lesions. These findings suggest that depression after right hemisphere lesions may have a different pathogenesis than mood changes after left hemisphere damage.

505.17

CAFFEINE SUPER-SENSITIVITY IN PANIC DISORDERS. E.M. DeMet, M.K. Stein*, C.K. Tran*, A. Chicz-DeMet. Dept. Psychiatry, Univ. of Calif., Irvine, CA. 92717.

Panic disorder (PD) patients are frequently sensitive to the anxiogenic effects of caffeine. These effects may be due to an antagonist action on adenosine receptors which attenuate excitatory neurotransmitters. The present study introduces a novel measure of adenosine receptor sensitivity and compares results obtained from normal controls with those from patients with PD and post-traumatic stress (PTSD). The test is based on a known action of adenosine receptors to potentiate the ability to taste quinine sulfate. Quinine taste thresholds were determined by a forced selection of trace concentrations from water standards. Adenosine receptor sensitivity was quantitated by comparing thresholds in the presence and absence of 10uM caffeine. Thresholds in the presence of caffeine were similar in the 3 subject groups. In contrast, PD patients had elevated baseline thresholds and larger difference scores than did controls or PTSD patients. The results are discussed in the context of a model whereby adenosine receptors upregulate in an attempt to control excitatory transmitter release, but this same mechanism confers caffeine supersensitivity.

505.18

D-AMPHETAMINE AFFECTS CENTRAL NORADRENERGIC MECHANISMS IN CHILDREN WITH ATTENTION DEFICIT-HYPERACTIVITY DISORDER (ADHD). B.A. Shaywitz, S.E. Shaywitz*, G.M. Anderson, P. Jatlow*, S.M. Gillespie*, B. Sullivan*, M.A. Riddle*, J.F. Leckman* and D.J. Cohen*. Yale University, New Haven, CT 06510.

Good evidence from many laboratories supports the belief that amphetamine (AMP) and methylphenidate (MPH) often produce remarkable ameliorative effects in children with ADHD, actions believed to occur via central catecholaminergic mechanisms. Despite their similarities, AMP and MPH may affect different catecholaminergic mechanisms. We employed a double-blind protocol to investigate 26 children with ADHD (24 boys, 2 girls) ages 5-14 years. Children received either AMP (.25 mg/kg, 11 children) or (0.5 mg/kg, 15 children) or placebo (n=26) and plasma MHPG and HVA were sampled:

	Hours after stimulant or placebo		
	4	5	6
Placebo	3.03±.13	2.88±.14	2.98±.15
MPH	2.83±.13	2.87±.14	3.16±.16
AMP	2.42±.12[a]	2.22±.12[b]	2.33±.15[c]

MHPG concentration (mean ± SEM) ng/ml

a: F=4.71, p=.014; b: F=3.25, p=.047; c: F=5.00, p=.011.

These findings suggest that despite their clinical similarity, d-amphetamine and methylphenidate differ in their effects on brain catecholaminergic systems with d-amphetamine influencing brain noradrenergic mechanisms, perhaps by reducing turnover of brain norepinephrine.

505.19

PRODUCTION OF NEGLECT IN RATS WITH UNILATERAL ABLATION OF VENTROLATERAL ORBITAL CORTEX. V. King*, J.V. Corwin, and R.L. Reep. Dept. of Psych., Univ. of New Orleans, New Orleans, LA 70148, and Depts. of Physio. Sci. and Neurosci., Univ. of Florida, Gainsville, FL 32610.

Neglect in humans and monkeys most often results from damage to cortical areas, including paralimbic cortex. Neglect in rodents has been produced by unilateral destruction of medial prefrontal cortex (PCm), a rodent analog of area 8 in primates. As with area 8, PCm has extensive connection with neocortical and paralimbic cortices, including bilateral reciprocal connections with the paralimbic area ventrolateral orbital cortex (VLO) (Reep, R.L. et al. 1984, 1987). The current study was conducted to determine if there is in rodents, as in primates, a cortical circuitry for directed attention by examining the effects of destruction of VLO.

Male Long-Evans hooded rats with lesions of right VLO (RV) (n=9), left VLO (LV) (n=9), and cortex just lateral to PCm (sham) (n=15) were tested for a minimum of four weeks on the following: 1) total neglect based on degree of orientation to visual, tactile and auditory stimuli; 2) extinction based on the tendency to touch the ipsilesional tab first when adhesive tabs were attached to each wrist; 3) motor asymmetry based on the difference between the number of ipsilesional and contralesional rotations during a two minute period.

No significant differences were found among the groups in rotational preference during the first 3 weeks. Although, both RVs and LVs showed significant total neglect relative to shams at week 1, the pattern of neglect within modalities differed between RVs and LVs. Whereas LVs showed significant neglect in the visual and auditory modalities versus shams at week 1, RVs were impaired on the tactile neglect measure, relative to shams at weeks 1 and 2 and on the extinction task versus shams and LVs at week 1, 2, and 3. The results indicate VLO may form part of a cortical circuitry for directed attention.

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505.20

LEARNING AND LATERALITY DIFFERENCES IN BXSB MICE AS A FUNCTION OF NEOCORTICAL ANOMALY. V.H. Denenberg, G.F. Sherman, G.D. Rosen, and A.M. Galaburda. Biobehavioral Sciences Graduate Program, U-154, Univ. of CT, Storrs, CT 06268; and Neuroanatomical Dyslexia Lab and Harvard Medical School, Boston, MA 02215

As part of a study relating immune factors, neural anomalies, and behavioral deficits, 23 BXSB mice were given a series of behavioral tests. Eight had ectopias in the frontal motor region (5M, 3F) while 15 did not (8M, 7F). On Collins' paw reaching test ectopic males used their left paws almost exclusively while ectopic females were equally strongly biased rightward. The normal males and females were scattered throughout (p <.04). Water escape learning is a simple spatial task requiring the animal to swim to a submerged platform. Mice with ectopias were faster, averaging 29.0 sec over five trials while mice with normal brains took 52.8 sec (p<.05). In a non- spatial discrimination learning task non-ectopic mice swam faster (p<.02) and made somewhat more correct choices (p<.11). Further analyses determined that non-ectopic males made more correct choices than ectopic males (p<.05),while the female groups did not differ.

Since ectopic animals swam faster in the water escape task but slower in the discrimination learning task, the learning differences cannot be due to motor factors that would influence speed of swimming. Some feature distinguishing the two tasks--such as spatial vs non-spatial, or choice vs no-choice--would appear to be associated with the presence of ectopias. In addition, left pawedness, maleness, and presence of ectopias were associated on the discrimination learning task.

505.21

THE CONE ELECTRODE: A LONG-TERM ELECTRODE THAT RECORDS FROM NEURITES. Philip R. Kennedy. Bioengineering Center, Georgia Institute of Technology, Atlanta, GA 30332.

A standard wire recording technique is combined with neurite growth into a piece of sciatic nerve to produce an electrode that records for months. The electrode is made by fixing a teflon insulated 3 mil gold wire to the inside of a 1.5 mm glass cone with a diameter of less than 200 microns at one end. Before implantation in deep layers of rat cortex, 5 to 10 fibers from the rat's sciatic nerve are placed inside the cone. The pin on the other end of the wire is cemented to the rat's skull.

For a few days after implantation no activity is evident. As neurites grow into the cone, activity builds up, first as background, then as single units. At week 4, many units appear so that multi-unit activity over 100 uVs in amplitude is recorded. When implanted into the vibrissa area of cortex, many vibrissa initially evoke activity, but from week 4 onwards, only 2 to 3 adjacent vibrissa evoke multi-unit responses. This suggests that a connection can be made between the cone electrode and functional areas of cortex. Histology at 3 months when still recording shows tissue growing into the cone holding it firmly in place. Retrograde dye labelling via the corticospinal tract shows neurites in the cone.

The cone electrode is expected to connect the central nervous system with augmentative devices in patients with severe communicative disorders.

BEHAVIORAL PHARMACOLOGY: MISCELLANEOUS

506.1

Discriminative Stimulus Properties of FG 7142. N.J. Leidenheimer and M.D. Schechter, Dept. of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

FG 7142 (N-methyl-β-carboline-carboxamide), a partial inverse agonist at the benzodiazepine receptor, produces both anxiogenic and proconvulsant effects. In the discriminative stimulus (D.S.) paradigm, FG 7142 produces drug-appropriate responding in DMCM and pentylenetetrazole trained rats. D.S. control has been established with FG 7142 and generalization to THBC and stressful environment manipulations has been reported. Furthermore, the FG 7142 D.S. is antagonized by benzodiazepine receptor agonists.

Male rats were trained to discriminate the effects of FG 7142 (5.0 mg/kg) in a food-motivated discriminative task. Dose-responsive generalization was observed following administration of the β-carbolines yohimbine (3.0-9.0 mg/kg) and norharmane (5.0-10.0 mg/kg) while coadminstration of FG 7142 with ZK 91 296 (15.0 mg/kg) resulted in antagonism of the FG 7142 D.S.. Rats trained to discriminate either yohimbine (3.0 mg/kg) or THBC (15.0 mg/kg) from vehicle failed to generalize to the FG 7142 D.S. and exposure to stressors resulted in vehicle responding. These results indicate that an asymmetrical generalization exists between FG 7142 and the β-carbolines THBC and yohimbine.

506.2

GABA-ERGIC MODULATION OF FELINE AGGRESSION ELICITED FROM THE MIDBRAIN PERIAQUEDUCTAL GRAY. M.B. Shaikh and A. Siegel. Dept. of Neurosciences, UMDNJ, Newark, N.J. 07103.

The midbrain periaqueductal gray (PAG) has been implicated in the initiation and regulation of aggressive behavior in the cat. Since the PAG is rich in GABA receptors, we examined the role of this putative transmitter in the modulation of affective defense (AD) and quiet biting attack behavior (QBA) elicited by electrical stimulation of the PAG.

Cannula-electrodes were employed for electrical stimulation as well as for microinjections of a GABA agonist (muscimol: 3, 12, 23, and 44 pmol/0.25ul) and GABA antagonist (bicuculline: 22 pmol/0.25ul). After establishing pre-drug response threshold values for AD and QBA, these drugs were microinjected into the PAG sites from which these responses were elicited. Microinjections of muscimol (12-44 pmol) significantly suppressed AD in a dose and time dependent manner. Pretreatment with bicuculline blocked the suppressive effects of muscimol (23 pmol) upon AD. In contrast, this dose of muscimol failed to alter the response threshold for QBA. Microinjections of vehicle alone (0.25 ul of saline, pH=7.4) did not modify the thresholds for either of these responses.

These results indicate that, at the level of the PAG, GABA-ergic mechanisms are selectively involved in the regulation of AD behavior in the cat.

[Supported by NIH Grant NS07941-19].

506.3

ANTAGONISM OF THE ANTI-CONFLICT EFFECTS OF PHENOBARBITAL, BUT NOT DIAZEPAM, BY THE A-1 ADENOSINE AGONIST l-PIA. T.C. McCloskey*, R.L. Commissaris*, G.M. Damian*, B. Brown*, R.A. Barraco and H.J. Altman (SPON: C.P. Spirito). College of Pharmacy & School of Medicine, Wayne State University, Detroit, MI 48202.

The present study examined the acute effects of the anxiolytics diazepam (DZ) and phenobarbital (PhB), the A-1-selective adenosine agonist N^6-R-phenylisopropyl adenosine (l-PIA) and the A-2-selective adenosine agonist 5-N-ethylcarbox amido adenosine (NECA) on behavior in the Conditioned Suppression of Drinking (CSD) paradigm, an "animal model" used to study anti-anxiety agents. Water-restricted rats were trained to drink from a tube that was occasionally electrified (0.5mA), electrification being signalled by a tone. DZ (1.25-10 mg/kg) or PhB (10-40 mg/kg) treatment resulted in a dose-dependent increase in punished responding, while neither l-PIA (15-250 nM/kg) nor NECA (2.5-20 nM/kg) produced a significant anti-conflict effect. In combination studies, neither l-PIA nor NECA pretreatment altered the effects of DZ. In contrast, pretreatment with l-PIA, but not NECA, reduced the anti-conflict effects of PhB. These data suggest that (1) neither A-1 nor A-2 adenosine receptor activation affects basal behavior in the CSD paradigm and (2) PhB, but not DZ, anti-conflict responses may result from interactions with A-1 adenosine receptors. (MH #42501-01; protocol conforms with NIH guidelines)

506.4

ALCOHOL-BENZODIAZEPINE RECEPTOR INTERACTIONS: AGGRESSIVE BEHAVIOR AND MOTOR ACTIVITY IN RATS AND SQUIRREL MONKEYS. E. Weerts*, W. Tornatzky* and K.A. Miczek. Dept. of Psychology, Tufts University, Medford, MA 02155.

The possible antagonism of ethanol's behavioral effects by the beta-carboline Zk93426 as compared to the imidazo-benzodiazepines Ro15-1788 and Ro15-4513, were studied in confrontations between resident and intruder rats as well as in interactions in group-housed squirrel monkeys. Quantitative ethological methods permitted the measurement of drug effects on elements of aggressive, defensive, submissive, and social behavior as well as on motoric activities. The low doses of ethanol (0.1, 0.3 mg/kg) enhanced frequency of agonistic behavior, while higher doses (1.0, 3.0 mg/kg) reduced their occurrence in both species. Zk 93426 (3.0 mg/kg), Ro15-1788 (10.0 mg/kg) and Ro15-4513 (1.0 mg/kg) did not antagonize the suppressive effects of ethanol in rats. Ro15-4513 and Ro15-1788 potentiated alcohol's sedative effects, and reduced agonistic behavior; Ro 15-1788 increased feeding, and Ro15-4513 induced tremors and seizures in squirrel monkeys when administered alone. However, Ro15-4513 reduced ethanol-induced staggering. These results indicate none of these substances completely block the biphasic effects of ethanol; yet the effects of Ro15-4513 on staggering behavior show some promise for identifying one element in the multiple mechanisms of action of ethanol.

506.5

NEURAL SUBSTRATES FOR LOCOMOTION PRODUCED FROM VENTRAL PALLIDAL STIMULATION. M.C. Austin and P.W. Kalivas. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164

It is well documented that nucleus accumbens efferents, which mediate locomotor behavior are GABAergic and project to the ventral pallidum/substantia innominata (VP/SI) region. The VP/SI region also contains a high density of enkephalin (ENK)-I.R. This study examines whether GABA-ergic or enkephalinergic neurons located in the VP/SI affects locomotor activity in rats. Intra-VP injection of either picrotoxin or bicuculline produced a dose-dependent increase in photocell counts with a minimum effect dose of 0.03 ug/side and 0.003 ug/side, respectively. To evaluate a possible interaction between GABA and Enk in the VP/SI, rats were pretreated with 0.1 ug/side (intra-VP) of the mu opioid antagonist naltrexone followed by injection of picrotoxin (0.1 ug/side). Naltrexone failed to attenuate the picrotoxin-induced hyperactivity. We also investigated the effect of intra-VP injection of the mu opioid agonist DAGO (Met-Enk analog). DAGO produced a dose-dependent increase in locomotor activity with a minimum effect dose of 0.01 nmoles/side. Pretreatment with 1.0 mg/kg s.c. of naloxone reversed the effect of DAGO (0.03 nmoles/side; intra-VP). These data indicate that inhibition of GABAergic transmission within the VP/SI produces a motor stimulant response which appears to be independent of ENK located in the VP. Furthermore, stimulation of enkephalinergic transmission in the VP/SI causes increased locomotor activity.

506.6

CENTRAL ADMINISTRATION OF BACLOFEN ENHANCES HABITUATION OF MOTOR ACTIVITY IN RATS. E.S. Sidel, H.A. Tilson, R.L. McLamb, H.S. Swartzwelder and W.A. Wilson. Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710, and Lab. Mol. Integ. Neurosci., NIEHS, NIH, P.O. Box 12233, Research Triangle Park, NC 27709.

The effect of baclofen, the β-p-chlorophenyl derivative of GABA, on consolidation was assessed using habituation to motor activity as a one-trial learning paradigm. Baclofen (3 nmol intracerebroventricularly, icv), administered immediately after a 15 minute training trial, significantly decreased motor activity upon re-testing 24 hours later. Neither the low dose of baclofen (1 nmol) nor saline vehicle alone exhibited this effect. When a delay of 10 or 60 minutes was inserted prior to injections, the effect was not evident. Baclofen (both 1 and 3 nmol) administered icv immediately prior to a 30 minute testing period significantly decreased activity when compared with saline-injected controls. This effect was no longer evident 24 hours later, indicating an absence of carry-over effects of the drug. In a one-trial step-through passive avoidance paradigm, baclofen (1 and 3 nmol) administered icv, did not significantly affect retention 48 hours later. One interpretation of these results is that when administered directly into the central nervous system, baclofen, a GABA b agonist, facilitates memory consolidation as measured under the conditions of these experiments. (This work supported in part by NSF grant RCD8651791).

506.7

DEFENSIVE BURYING BEHAVIOR IN MAUDSLEY REACTIVE (MR/Har) AND NON-REACTIVE (MNRA/Har) RATS. S.L. Beardslee*, E. Papadakis*, H.J. Altman, G.M. Harrington* and R.L. Commissaris* (SPON: E.P. Schoener). College of Pharmacy & School of Medicine, Wayne State University, Detroit, MI 48202 and Dept. Psychol., U. Northern Iowa, Cedar Falls, IA 50614.

Based upon differences in open field and conflict behaviors, the MR/Har and MNRA/Har rat strains have been proposed as a genetically-based "animal model" for the study of emotionality and/or anxiety. The present study compared the MR/Har and MNRA/Har rat strains in the Defensive Burying (DB) paradigm described by Treit (Br. Res. Bull. 19:401-404, 1987). After four daily habituation sessions, female rats were placed in the DB chamber singly. Subjects received a 3 mA shock upon contact with a wire-wrapped prod and were observed for burying behavior (movement of the bedding material toward or over the prod) for 15 minutes post-shock. Although MR/Har rats tended to initiate burying sooner and exhibit a longer duration of burying than did the MNRA/Har rats, there were no differences in the frequency of subjects exhibiting burying behavior in MR/Har (12/14) as compared to MNRA/Har (11/13) rats. Thus, although they differ dramatically in open field and conflict behaviors, the Maudsley rat strains do not differ on all measures of DB behavior. (MH #42501-01) Protocol conforms with NIH Guidelines.

506.8

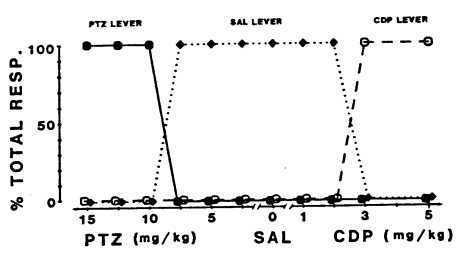
A COMPARISON OF CHLORDIAZEPOXIDE AND BUSPIRONE IN THE SHOCK-PROBE/BURYING TEST FOR ANXIOLYTICS. D. Treit and M. Fundytus*. University of Alberta, CANADA T6G 2E9.

The effects of chlordiazepoxide (2.5-10.0 mg/kg IP) and buspirone (0.05-1.0 mg/kg SC) were compared by a "blind" observer in the shock-probe/burying test for anxiolytics. Consistent with their anxiolytic effects, both agents decreased rats' burying behavior, and increased the number of shocks rats received from a constantly electrified (2 mA) shock probe, at doses that did not affect rats' general activity. The suppression of probe-burying and facilitation of probe-shocks increased linearly as a function of drug dose, with the relative potency of buspirone being substantially greater than that of chlordiazepoxide. These results contrast with those of Craft et al. and suggest that their failure to show a selective suppressive effect of chlordiazepoxide and buspirone on burying to a constantly electrified shock-probe was due to inappropriate methodology (e.g., lack of distributed pretest habituations, absence of separate placebo control groups, and a behaviorally sedating range of anxiolytic doses). In any case, under the parameters used in the present study, this "repeated shock" version of the shock-probe test for anxiolytics appears to be more efficient than the previous, single shock version (e.g., only 2% subject "attrition" compared to 20%-40%), and current studies are underway to further characterize its drug-class specificity.

506.9

THREE-CHOICE DRUG DISCRIMINATION OF AN UNIDIMENSIONAL "ANXIOGENIC/ANXIOLYTIC" CONTINUUM. D.V. Gauvin*, and F.A. Holloway. (SPON: H.D. Christensen) Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.

To lend support to our previous suggestion that the interoceptive states induced by chlordiazepoxide (CDP) and pentylenetetrazol (PTZ) lie at polar ends along a single affective continuum (Michaelis et al., Psychopharmacology, in press, 1988), 6 Sprague-Dawley rats were trained in a 3-choice drug discrimination task utilizing CDP (5 mg/kg), saline (SAL), and PTZ (15 mg/kg) as discriminative stimuli. Average sessions to 90% criterion was 102 ± 5. Data from one representative subject is shown below. Generalization tests resulted in pharmacologically specific, quantitative, unidimensional functions.



506.10

ENHANCEMENT OF OPIOID CATALEPTIC RESPONSE BY CORTICAL FRONTAL DEAFFERENTATION OR INTRASTRIATAL INJECTION OF NMDA-RECEPTOR ANTAGONISTS. S. Consolo*, G.L. Forloni*, H. Ladinsky and E. Palazzi*. Mario Negri Institute, Milan, Italy.

The cataleptic activity of morphine and methadone was markedly potentiated in decorticated rats with no changes in the onset or duration of action. Enhancement of opioid catalepsy was not due to changes in the availability of the drugs in the brain. The potentiation of methadone induced catalepsy in decorticated rats was mimicked in naive rats by intrastriatal (i.s.) injection of AP7, a selective antagonist of NMDA receptors. The failure of AP7 to elicit an effect after injection into the n. accumbens indicates a selective involvement of the striatum in the phenomenon. That the striatum plays a critical role in opioid-induced catalepsy was substantiated by the findings that: 1) naloxone (i.s.) prevents the potentiation of catalepsy induced by methadone and morphine in decorticates, 2) oxotremorine (i.s.) reverses the enhancement of opioid catalepsy in decorticates. In conclusion, evidence is given that the corticostriatal pathway exerts an inhibitory effect upon narcotic-induced cataleptic behavior. (Supported by AFOSR-87-0399).

506.11

BLOCKADE OF THE ACOUSTIC STARTLE REFLEX BY LOCAL INFUSION OF EXCITATORY AMINO ACID ANTAGONISTS INTO THE VENTRAL COCHLEAR NUCLEUS. M.J.D. Miserendino* & M. Davis (SPON: J. ROSEN) Yale Univ., Dept. of Psychiatry, Ribicoff Res. Fac., Conn. Mental Health Ctr., New Haven, CT. 06508.

A large body of data suggests that the excitatory amino acids glutamate and/or aspartate may be the neurotransmitters released by the auditory nerve at the cochlear nucleus. We are using the acoustic startle reflex to behaviorally examine the role of excitatory amino acids and their receptors in auditory transmission at the level of the ventral cochlear nucleus (VCN), the first synaptic relay in the neural pathway mediating acoustic startle.

Rats were cannulated in one VCN and received an electrolytic lesion of the contralateral VCN. Because this surgical procedure itself decreases startle amplitude, all animals were pretreated with the phosphodiesterase inhibitor rolipram (1 mg/kg, ip) which has been shown to elevate startle. Ten minutes later, rats were infused with either 5, 25, or 50 nmols of gamma-D-glutamylglycine, a potent non-specific excitatory amino acid antagonist, or with artificial cerebrospinal fluid (ACSF). Startle elicited by 95dB, 50 msec noise bursts was measured over the next 40 minutes.

ACSF had no effect on startle. In contrast, gamma-D-glutamylglycine caused a dramatic, dose-dependent depression of the startle reflex, with the highest dose producing a complete blockade of startle. These results support the idea that excitatory amino acids are involved in startle at the level of the ventral cochlear nucleus, and illustrate that the acoustic startle reflex is a sensitive behavioral assay of VCN receptor function. Current work utilizing more specific NMDA and non-NMDA antagonists will attempt to characterize the amino acid receptor subtype(s) in the VCN mediating the acoustic startle reflex.

506.12

DIFFERENT TEMPORAL EFFECTS OF MK-801, PCP AND KETAMINE ON MEMORY RETENTION IN MICE. M.J.Benvenga, A.V.Wing*, and T.P.Jerussi. Anaquest/BOC Health Care, Murray Hill, New Jersey, 07974.

MK-801, a novel anticonvulsant, has recently been reported as the most potent ligand of the PCP receptor in vitro (Sircar, et.,al., Brain Res, 1987). MK-801 also inhibits passive avoidance retention in vivo (Benvenga & Spaulding, Pharm Biochem Behav, 1988). The purpose of the present experiment was to evaluate the effect of MK-801 given pre- and posttraining in a passive avoidance task and compare these results with phencyclidine and ketamine.

Male Swiss-Webster mice (25-30g) were injected either 30 minutes prior to or immediately following one-trial passive avoidance training (N=8/group) which consisted of footshock delivered to the animal when it entered a darkened chamber. Groups were treated with PCP (0 [vehicle = distilled water], 0.5, 0.75, 1.0, 2.0 or 5.0 mg/kg.), ketamine (0, 2.0, 5.0, 7.5, 10.0 or 15.0 mg/kg.) or MK-801 (0, 0.05, 0.1, 0.15, 0.75, 1.0 or 1.5 mg/kg.). All mice were tested 24 hours after initial exposure to the test apparatus and the latency to reenter the darkened chamber was recorded. Median group latencies were compared by Mann-Whitney U-tests.

The results indicated that all drugs tested produced an inhibition of retention in the passive avoidance task. However, MK-801 produced a posttraining effect only at 10 times the dose necessary to cause a pre-training effect while PCP and ketamine produced posttraining effects at 2 times the pretraining dose. One possible mechanism for this effect may be that PCP interacts with endogenous opioidergic and cholinergic systems whereas MK-801 does not.

506.13

EFFECTS OF CHRONIC NIMODIPINE TREATMENT ON BEHAVIOR OF OLD RATS

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Growing evidence ascribes an important role in aging processes in the brain to a dysregulation of the Ca^{2+}-homeostasis in neurons. Compounds which interfere with Ca^{2+}-fluxes such as Ca^{2+}-entry blockers might therefore possess a therapeutic potential in age related brain disorders. Nimodipine, a Ca^{2+}-blocker of the dihydropyridine type, was investigated after subchronic and chronic treatment for its ability to improve behavioral deficits occuring in old rats such as impaired learning and memory capacity, reduced open field activity and social behavior, impaired motor coordination etc. Nimodipine treatment for one week improved the learning rate of old rats in a water maze. Rats chronically fed with nimodipine containing food showed a higher level of exploration in an open field than normally fed old rats. Furthermore, motor coordination of nimodipine fed animals was significantly better than that of age-matched controls. Chronic nimodipine treatment also delayed the onset of age-related walking patterns in old rats as measured by analysis of the foot prints in an alley-walking test.

The present results indicate that nimodipine may be an useful drug for the treatment of certain aspects of brain aging and support recent findings obtained from clinical studies with aged people treated with nimodipine.

506.14

OXYTOCIN RECEPTORS SENSITIZE BRAIN TO VASOPRESSIN'S CONVULSIVE ACTIONS. P.P.Poulin and Q.J. Pittman. Neuroscience Research Group, Univ of Calgary, Calgary, Alberta T2N 4N1 Canada.

Vasopressin (AVP) administered into the ventral septal area (VSA) of rats produces severe motor disturbances which appear to result from an interaction of AVP with V1 type of AVP receptors and appear to involve a sensitization process whereby a first administration (sensitization) of AVP causes minor motor disturbances, while a second dose two days later causes sever motor disturbances. In binding studies, oxytocin (OT) has been shown to bind to VSA V1-like AVP receptors (Poulin et al., J. Neurochem. 50(3)889-897), with 100 times less potency than AVP. Thus we have investigated whether OT could sensitize or induce motor disturbances in rats after intracerebroventricular (icv) injections. Dose response studies show that AVP can sensitize and induce motor disturbances at threshold doses of 10 ng and 1 ng icv respectively. OT (1 ng icv) was 10 times more potent that AVP (10 ng icv) in sensitizing the rat brain to AVP (1 ng icv) induced motor disturbances. In AVP or OT sensitized animals however, OT (100 ng), was ineffective in inducing motor disturbances at 100 times AVP (1 ng icv) effective dose. Thus, it is possible that AVP or OT sensitization effects, but not AVP induced motor disturbances, are mediated via an oxytocin type receptor which also recognizes AVP. Supported by MRC. P.P. is an AHFMR student.

506.15

SELECTIVE ENHANCEMENT OF LATENT INHIBITION BY ANTIPSYCHOTIC DRUGS IN THE RAT:A POSSIBLE MODEL FOR CLINICAL ACTION. L.A. Dunn*, G.E. Atwater*, G.W. Christison*, C.D. Kilts*,(SPON:R.D. Weiner) Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

Latent inhibition (LI) of conditioned responding is a quantitative measure of the ability to screen out irrelevant sensory stimuli. This ability has been shown to be deficient in schizophrenia patients and to improve with antipsychotic medication. We have assessed the ability of several classes of psychoactive drugs to enhance LI in rats using an established paradigm (Christison et al., Biol. Psychiat., 23:746-749, 1988). All drugs were given i.p. for 7 days. Following the final injection, drug and vehicle treated rats were divided into groups and placed in darkened test cages. Half were preexposed 20 times to a 28v, 40 mA cage light. All groups were conditioned with 2 CS-US pairings. The CS was identical to the pre-exposure stimulus. The US consisted of a 1 sec, 0.75 mA scrambled footshock. The following day, conditioned emotional response (CER), as measured by interruption of drinking in response to the CS, was recorded. Statistical significance was evaluated using a 2x2 ANOVA for each experiment. LI was enhanced by treatment with haloperidol 0.3mg/kg (p=0.008), and fluphenazine 0.3mg/kg (p=0.026). Clozapine 10mg/kg, imipramine 10mg/kg and chlordiazepoxide 10mg/kg showed no enhancement of LI. A further experiment in which clozapine 10mg/kg was given only once prior to preexposure also failed to show LI enhancement. The fact that clozapine did not enhance LI may indicate that this paradigm is sensitive to typical but not atypical antipsychotics. (MH39967)

506.16

POLYAMINES, EXPLORATORY ACTIVITY AND SENSATION-SEEKING. P.A. Ferchmin, V.A. Eterović, J.R. Cotto Aponte*, E.M. Rivera*, W.I. Rodríguez*, and S.A. Alvareztorre*, Dept. of Biochemistry, Univ. Central del Caribe, Cayey, P.R. 00633 and Interamerican Univ. San Juan, Puerto Rico, USA.

We have studied the effect of drugs which affect brain polyamine levels on rat activity in a Greek cross-shaped maze, with two white and two black compartments, communicated with a central gray compartment. Total entries into peripheral compartments measure exploratory activity, while white entries are a measure of sensation-seeking behavior. Thirty day-old, male, albino rats were used. Difluoromethylornithine (DFMO), 400 mg/Kg, decreased brain putrescine (Pu) to 50% of normal level; other polyamines (PA) were not affected. DFMO did not affect white entries but depressed total entries. Exogenous Pu, 400 mg/Kg, increased brain Pu level to six times normal 15 min after injection, declining slowly afterwards. Spermidine and spermine levels were not affected, and acetylated PA were undetectable. Pu injection depressed white entries and this effect was reversed by DFMO. Pu also depressed entries to black compartments (and therefore total entries), but this effect was not reversed by DFMO. Cyclohexylamine, 400 mg/Kg, which increase endogenous Pu, depressed total, but not white entries. In summary, depression of total entries was observed with any disturbance of Pu level, while white entries were only affected by exogenous Pu.

(Supported by NIH-MBRS RR08159 and NIH-RCMI RR08102)

506.17

INVOLVEMENT OF ENDOGENOUS MELATONIN IN THE ANTIDEPRESSANT-LIKE ACTIVITY OF LUZINDOLE (N-0774). Areso, P.*, and Dubocovich, M.L. (SPON: R.S. Eisenberg). Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The selective melatonin receptor antagonist luzindole (LUZ) exerts antidepressant-like activity in the C3H/HeN mice behavioral despair test (Mogilnicka & Dubocovich, Neurosci. Abs. 13:1039, 1987). Here, we further investigated the involvement of melatonin (MEL) in the behavioral despair test using LUZ. The time of immobility (sec) during swimming was determined in the dark phase of a 14:10 h, L:D cycle in C3H/HeN mice which synthesize MEL and in C57BL/6 mice which do not synthesize the hormone. In controls, the duration of immobility was 59 ± 5 (n=47) in C3H/HeN and 147 ± 4 (n=24) in C57BL/6. Desipramine (30 mg/kg, i.p.) significantly reduces the duration of immobility both in C3H/HeN (3 ± 1, $p<0.001$, n=16) and in C57BL/6 (104 ± 18, $p<0.001$, n=8). LUZ (10 mg/kg, i.p.) reduces the duration of immobility in C3H/HeN (17 ± 3, $p<0.001$, n=31) but not in C57BL/6 (170 ± 3, n=24). While MEL (30 mg/kg, i.p.) did not affect the time of immobility in C3H/HeN (52 ± 5, n=24) it reversed the anti-immobility effect of LUZ (57 ± 9, n=15). The lack of effect of LUZ in C57/BL6 mice and the antagonism of its effect by MEL in C3H/HeN mice suggest an involvement of endogenous MEL in the behavioral despair test. It is suggested that the antidepressant-like activity of LUZ occurs through a different mechanism than that of classical antidepressants.
Supported by DK-38607 and Nelson Research.

506.18

DISCOVERY OF A CANNABINOID RECEPTOR IN RAT BRAIN.
W.A. Devane*, L.S. Melvin*, M.R. Johnson* and A.C. Howlett (SPON: M.M. Voigt). Dept. of Pharmacology, St. Louis Univ., St. Louis, MO 63104 and Pfizer Central Research, Groton, CT 06340.

CP-55,940 is a nonclassical cannabinoid analgetic agent whose ability to inhibit adenylate cyclase in a neuronal cell model has recently been reported (Howlett et al., Mol. Pharm. 33:297, 1988, cmpd 8). ^{3}H-CP-55,940 was used to characterize a receptor site in brain. Using a P2 preparation from rat cortex, a binding site was characterized which was saturable with either 1 μM CP-55,940 or delta-9-tetrahydrocannabinol. Cannabinol and cannabidiol, cannabinoid drugs lacking CNS activity, did not displace CP-55,940. Scatchard analysis of saturation curves indicated a high affinity site having a K_d of 110 pM and a lower affinity site having a K_d of 1.4 nM. K_d values calculated from the association and dissociation rates were similar. Guanylylimidodiphosphate eliminated the binding to the high affinity site, and increased the number of low affinity sites, indicating that a characteristic interaction of the receptor with G-proteins is likely. Divalent cations increased binding, and Na and other monovalent cations decreased binding of CP-55,940. This behavior is characteristic of other receptors associated with inhibition of adenylate cyclase.
(Supported by DA03690, NS07254, and NS00868.)

NEUROTOXICITY: STUDIES IN TISSUE CULTURE

507.1

CALBINDIN D-28K PROTECTS AGAINST GLUTAMATE INDUCED NEUROTOXICITY IN RAT CA1 PYRAMIDAL NEURON CULTURES. K.G. Baimbridge and J. Kao*. Dept. Physiology, University of British Columbia, Vancouver, B.C., Canada. V6T 1W5

When neuronal cultures of rat CA1 pyramidal cells are exposed briefly to high levels of glutamate a delayed, (24hr), and Ca^{++} dependent neuronal death occurs. Depending upon the glutamate concentration, however, some neurons do survive. We have now measured both resting and glutamate stimulated intracellular Ca^{++} concentrations using neurons loaded with Fura II, and have correlated these values with the presence or absence of Calbindin D-28K, (CaBP).

Stimulation of the neurons with glutamate over the range 0.1-100 uM produced a dose dependent increase in the Ca^{++} levels. At the highest dose the range of Ca^{++} clearly fell into two distinct groups with mean Ca^{++} levels of 500 or 2000 nM. Of the 100 neurons measured 26 were CaBP positive as determined immunohistochemically (ICC) immediately after the glutamte insult. All of these CaBP positive neurons had Ca^{++} levels in the lower group range. When CaBP ICC was done on surviving neurons 24 hours following the glutamate insult the percentage of CaBP-positive neurons doubled when compared to non-stimulated controls. We suggest that these results are consistent with a calcium-buffering role for CaBP which may buffer the glutamate induced rise in intraneuronl Ca^{++} and as a result limit the effectiveness of the glutamate induced delayed neurotoxicity.

507.2

PHOTOLYTIC EFFECT OF POLYLYSYL-PHTHALOCYANINE DERIVATIVES ON MURINE NEURO 2a NEUROBLASTOMA CELLS. S. Kornguth, F. Sieber* and T. Kalinke*. University of WI, Madison WI 53706 and Med. Coll. WI, Milwaukee WI.

Poly-L-lysine (PL) was modified covalently with tetracarboxy-cobalt-phthalocyanine (Pc) using varying ratios of Pc per lysyl residue. The effect of incubating murine neuroblastoma neuro 2a cells with the PL-Pc complexes was examined as a function of duration of light exposure and the degree of modification of the PL with the Pc. The PL-Pc complexes are of interest because: a) PL binds many tumor cells more avidly than normal cells, b) Pc is taken up more rapidly by tumor than by normal cells, c) cells that bind Pc are damaged after exposure to light, d) the paramagnetic cobalt of Pc may serve as a MRI probe to detect cellular uptake of Pc, e) Pc has a high molar extinction coefficient. In our experiments, $6x10^5$ cells were incubated in 2 ml of HEPES buffered medium containing 14% fetal bovine serum with polylysine alone or with PL-Pc (between 0.1 to 1.0 mg/ml). Two preparations of PL-Pc were used (10 lysyl residues per Pc and 30 lysyl residues per Pc). The cells were incubated with the PL or with the PL-Pc under three conditions (in the dark for 90 min.; in the dark for 30 min and then in light for 30 or for 60 min). The cells were then washed and cultured in methyl cellulose for 8 days. The clonogenic assay revealed that polylysine alone was toxic to the cells and that the PL-Pc treatment resulted in photolysis of the neuro 2a cells. These data suggest that the PL-Pc may be useful in the treatment of some tumors.

507.3

EFFECTS OF HALOTHANE ON MICROFILAMENTS IN CULTURED RAT FIBROBLASTS. E. Uemura, S. Jeftinija and D. Gledic. Dept. of Vet. Anatomy, Iowa State University, Ames, IA 50011.

To examine the mechanism of halothane toxicity that was characterized by the suppression of axonal and dendritic extension, actin distribution in fibroblasts in vitro, was studied by immunocytochemistry using the anti-biotin peroxidase complex technique. Rat fibroblasts were cultured in the presence of 1% halothane in gas phase up to 41 hours. The growth rate of the cell was significantly slowed down by halothane. Although halothane did not affect the size of cells, it affected the distribution pattern of actin. The control cells were characterized by the deeply stained mesh-work of actin throughout the cytoplasm. However, the cells that were exposed to halothane showed diffusely stained actin confined to the perinuclear area, and a large portion of the cytoplasm was not stained. Analysis of staining intensity of cells by an image analyzer clearly supported such qualitative observation. It appears that the effect of halothane on neuronal extension is a reflection of the microfilaments inability to function in the presence of halothane.
Supported by the March of Dimes Birth Defects Foundation (grant no. 15-56).

507.4

MEASUREMENT OF INTRACELLULAR CALCIUM ION CONCENTRATIONS IN NG108-15 CELLS BY FLUORINE-19 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY: A MODEL FOR THE STUDY OF LEAD NEUROTOXICITY.
F.A.X. Schanne*, T.L. Dowd*, R.K. Gupta* and J.R. Moskal (SPON:A. Herschfeld). Albert Einstein College of Medicine, Bronx, New York 10467.

Intracellular Ca ion concentrations $[Ca^{2+}]_i$ were measured in NG108-15 cells using the intracellular divalent cation indicator 1,2-bis(2-amino-5-fluorophenoxy)ethane-N,N,N',N'-tetraacetic acid (5F-BAPTA) and fluorine-19 Nuclear Magnetic Resonance Spectroscopy (NMR). This methodology provides for the simultaneous identification and measurement of $[Ca^{2+}]$ and a variety of heavy metals, including $[Pb^{2+}]$. The NG108-15, a hybrid cell line of murine neuroblastoma x rat glioma, makes synapses upon chemically induced differentiation and co-culturing with fetal rat myotubes. NG108-15 cells were grown on Cytodex 1 microcarriers (Pharmacia) in Dulbecco's Modified Eagle's Medium (GIBCO) supplemented with 10% fetal bovine serum. Cells were loaded with 5F-BAPTA and superfused at 2 ml/min with oxygenated medium during NMR observation. NMR measurements were performed on a Varian VXR 500 using a 10 mm broad band probe tuned to 470 MHz for fluorine. Using this methodology the average $[Ca^{2+}]_i$ was measured to be 105 nM. Thus, NG108-15 cells will be used as a model to study the effects of Pb on the early events leading to synaptogenesis, specifically as it affects $[Ca^{2+}]_i$.

507.5

MODULATION OF MPP+ NEUROTOXICITY IN THE N_2AB-1 NEUROBLASTOMA CELL LINE. S. J. Simmons and M. F. D. Notter. Environ. Health Sci. Ctr. and Dept. of Neurobio. and Anat., Univ. of Rochester Sch. of Med., Rochester, NY 14642.

A mouse adrenergic neuroblastoma cell line (N_2AB-1) was used to investigate toxicity of 1-methyl-4-phenylpyridinium iodide(MPP+), the active metabolite of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine(MPTP) in an in vitro model. Cells were differentiated by prostaglandin E_1 and dibutyryl cyclic AMP to induce a neuronal phenotype. Both differentiated and mitotic cells were exposed to MPP+(3.4 - 340μM) for 24-72 hr.

Differentiated cells were less sensitive to or protected from MPP+ toxicity as compared to mitotic cells. Toxic response was seen morphologically as increased cytoplasmic inclusions, loss of neurites, loss of intact cells and cell death. Measurement of cell number over the 3 day observation period confirmed the morphologic findings of protection with differentiation.

To further explore the benefits of differentiation, cells were treated with mixed gangliosides(GA)(200μg/ml), a neurite promoting factor in the CNS. GA have been shown to aid in neuronal recovery in vivo from MPTP toxicity(Hadjiconstantinou M. et al., Neuropharm. 25:1075, 1986). GA induced neurite formation in both mitotic and previously differentiated cells. 24 hr pretreatment or cotreatment of GA with MPP+ reduced the loss of [3H]-leucine incorporation during protein synthesis and prevented the loss in total protein induced by MPP+ in mitotic and differentiated cells. GA aided in the recovery of both mitotic and differentiated cells following removal from MPP+ exposure. Recovery included cell proliferation and the extension of neurites.

This in vitro model of neurotoxicity provides a system in which agents can be tested for their ability to protect against a neurotoxin.

Supported in part by NIEHS Pilot Grant from ES01247.

507.6

BIOCHEMICAL AND ULTRASTRUCTURAL MODIFICATION IN PC12 CELLS TREATED WITH "PARKINSON MIMETIC" TOXINS. A. Vescovi*, M. Gebbia*, M. Mora*, G. Cappelletti*, E. A. Parati. (SPON: L. M. Vicentini). Inst. "C. Besta" Milan, Italy, 20133.

We studied the effects at different incubation time of 1.5mM MPTP or MPP^+ or $MnCl_2$ on PC12 cultured cells. We analized cellular death, intracellular levels of dopamine and dopac and ultrastructural morphology. Data show that within 3 days neither MPTP nor $MnCl_2$ affected cells survival, while after 24 hours MPP^+ led to a complete cells death (97%). A strong difference between manganese and MPTP was observed at biochemical level; after 3 hours 1.5 mM MPTP induces a decrease in dopamine and dopac intracellular levels (-80%), while 3 hours of 1.5m manganese chloride treatment results in an increase of about 120 %, followed by a slow decrease reaching a value of 56% of untreated cells after 24 hours. These biochemical data seem to be confirmed by ultrastructural observations, considering that despict the unaltered vitality, PC12 cells show signes of sufferring, with an intracellular accumulation of lipofuscin-like material in both MPTP and $MnCl_2$ treated cells. Our observations suggest that although both manganese and MPTP induce similar anatomopathological damages in man and primates, at least in the earlier stages of intoxications the biochemical mechanisms involved seem very different, inducing opposite neurochemical modifications.

507.7

ALUMINUM NEUROTOXICITY IN CULTURED NG108-15 CELLS. H.S. Singer*, C. Searles*, J.L. March§*, and J.C. Troncoso§. Dept. of Neurology and §Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182.

In order to better understand the pathogenesis of neurofilament (NF) and neurotransmitter changes in aluminum (AL) neurotoxicity, we have begun to explore the effects of AL lactate on cell cultures. Rapidly dividing neuroblastoma x glioma hybridoma (NG108-15) cells, passages 24-28, were cultured for up to eight days in concentrations of AL lactate (0.5-4 mM). Cell growth, as determined by total protein content, was not reduced until AL in the media exceeded 2 mM. In the presence of 1-2 mM AL, the activity of choline acetyltransferase (ChAT) was increased (132% of control), whereas glutamate decarboxylase (GAD) activity was unchanged from control. Saturation kinetics showed an increased amount of ChAT with a stable affinity. At 4 mM Al, both ChAT and GAD activities were reduced (85% and 33%, respectively). Alterations of NF were not identified with either light or electron microscopy, even after exposure to the highest AL concentrations. Analysis of proteins separated by gel electrophoresis also failed to document any increase in NF polypeptides. AL neurotoxicity is a complex phenomenon known to affect both synaptic neurotransmission and cytoskeletal proteins. The NG108-15 cell line is a useful in vitro model for further characterization of the effect of AL on the cholinergic system.

507.8

INHIBITION OF NEURITE GROWTH BY ORGANIC AND INORGANIC LEAD. G. Audesirk, G. Nelson,* D. Shugarts* and J. Przekwas*. Biology Department, University of Colorado at Denver, 1200 Larimer St., Denver, CO 80204.

We investigated the effects of inorganic lead and triethyl lead on neurite growth in primary cultures of neurons from the brains of pond snails (Lymnaea stagnalis) and chick embryos. Neurons were cultured for 3 to 4 days in medium that contained no toxins, inorganic lead ($PbCl_2$), or triethyl lead chloride. Lymnaea neurons were scored for neurite growth, with a neurite defined as a process at least one soma diameter in length. Inorganic lead reduced the fraction of cells that grew neurites, with an IC_{50} of about 13 uM. Triethyl lead reduced the fraction of cells growing neurites with an IC_{50} of about 0.5 uM, and exerted significant toxicity at 0.2 uM. Neurite length was not measured. In chick neuron cultures, the neurites of all the neurons in randomly selected areas of the culture dishes were measured with a digitizing tablet. Inorganic lead reduced the fraction of neurons that grew neurites (IC_{50} of 226 uM), but did not reduce the mean neurite length per neurite-growing cell. Triethyl lead reduced both the fraction of neurons growing neurites (IC_{50} = 0.25 uM) and the mean neurite length (IC_{50} = 0.42 uM).

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507.9

EFFECT OF MPTP ON PRIMATE ADRENAL CHROMAFFIN CELLS IN VITRO: AN ACUTE AND CHRONIC STUDY. M. Kaniucki*, J.T. Hansen, M.S. Fiandaca, J.H. Kordower, D.M. Gash, S. Okawara, and M.F.D. Notter. (SPON: L. Abood). Dept. Neurobiol. and Anat., and Div. of Neurosurg., Univ. of Rochester, Rochester, New York 14642.

Primate adrenal medullary cells were exposed to 1 methyl 4 phenyl 1,2,3,6 tetrahydropyridine (MPTP) in vitro to examine the effect of this neurotoxicant on peripheral catecholamine (CA)-containing tissues. Chromaffin cells obtained from either monkey or the Human Organ Donor Program at the University of Rochester were enriched by differential plating and cultured in the presence or absence of nerve growth factor (NGF, 100 ng/ml). After one week, cells were exposed to 150 uM MPTP for an additional week. Cells which had extended neurites in the presence of NGF showed no morphological effect of MPTP at the light microscopic level. However, there was a significant loss in CA as seen by histofluorescence and high performance liquid chromatography (HPLC). Electron microscopy revealed a depletion in dense core vesicles after chronic exposure to MPTP. Replacement of MPTP medium with standard medium stimulated a restoration of CA histofluorescence after seven days. An acute 15 min pretreatment of chromaffin cells with MPTP or its active metabolite methylpyridinium ion (MPP+) induced a dose dependent secretion of CA over a one hour pulse with MPP+ (150 uM) producing the maximum and most rapid secretion as determined by HPLC. Exposure of chromaffin cells to these neurotoxicants did not affect the physiological response to acetylcholine (10-9 M) as all cultures showed a stimulated release of CA. These data indicate that MPTP induces a dramatic loss in catecholamines in primate chromaffin cells in vitro after both acute and chronic exposures, while CA can be restored to chromaffin cells following removal of this toxic agent without effect on the integrity or physiological function of these cells.

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507.10

AFFECTS OF MPTP ON A HUMAN ADRENERGIC CELL LINE, LA-N-1. S. Sullivan and M.F.D. Notter. Univ. of Rochester Sch. of Med., Dept. of Neurobiology and Anatomy, Rochester, NY 14642

The effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were studied on a human neuroblastoma cell line in vitro before and after differentiation. LA-N-1 cells, which are neural crest derivatives, are adrenergic and differentiate morphologically and biochemically when treated with 10^{-5}M retinoic acid (RA), a vitamin A analog.

Mitotic cultures exhibited a dose dependent decrease in cell number after treatment with 1, 10 or 50 μg/ml MPTP (4.77, 47.7 or 238.5 μM respectively). Cultures treated with 1 μg/ml had similar growth curves to control cultures. Cultures treated with 10 μg/ml showed a 35% decrease in cell number by day 2 of treatment; this decrease remained throughout the 5 day test period. Cultures treated with 50 μg/ml exhibited an 85% decrease in cell number by day 2 and continued to decline to a 99% decrease by day 5.

Cell numbers in differentiated cultures were less affected by MPTP treatment. The growth curves of cultures treated with 1 and 10 μg/ml were similar to controls. However, treatment with 50 μg/ml was still acutely toxic, causing a 70% decrease in cell number which remained constant from day 2 until day 5.

Although cell numbers were not severely affected by low doses of MPTP there were apparent morphological changes in differentiated cultures. Differentiation with RA causes cells to stop dividing and to send out long neuritic processes. MPTP caused a dose dependent "dedifferentiation." Cells retracted their neurites and also changed in size and shape.

These data indicate that this human neural cell line is a good in vitro model of MPTP toxicity and that early changes seen in vitro may reflect long term effects seen in vivo.

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507.11

EFFECT OF LEAD AND MERCURY ON LIPID METABOLISM OF SCIATIC NERVES IN VITRO AND CULTURED SCHWANN CELLS.

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The goal of this study was to analyse the action of lead and mercury on the lipid metabolism of Schwann cells in different states of functional differentiation.

In the first series of experiments, endoneuria of sciatic nerve from mice were preincubated at 37°C in the presence of various concentrations of lead nitrate or mercuric chloride. After 30 minutes preincubation, radiolabeled acetate or acetoacetate was added as precursors for lipid synthesis and the mixture was incubated for 2 hours. In a second set of experiments, Schwann cells isolated from sciatic nerves of 4 old-day mice were cultured for 15 days, then incubated 18 hours at 37°C with various concentrations of lead or mercury in the presence of one of the same precursors. Lipids were then extracted, separated, and level of the radioactivity was measured.

Lead did not affect lipid metabolim of endoneurium and Schwann cells in culture, consistent with the hypothesis that Schwann cells are resistant to low concentrations of lead. 10^{-5}M $HgCl_2$ caused an increase in the incorporation of acetate into phospholipids of endoneurium and modified the pattern of acetate incorporation into lipids of both endoneurium and Schwann cells in culture. With acetoacetate as precursor, 10^{-5}M $HgCl_2$ modified only the pattern of its incorporation into lipids of endoneurium. These results suggest that the effect of mercury on lipid metabolism in Schwann cells depends on the state of functional differentiation of these cells.

507.12

NEUROTOXIC EFFECTS OF METHAMPHETAMINE ON DOPAMINE AND SEROTONIN NEURONS IN REAGGREGATE TISSUE CULTURE. P.J. Kontur, L.A. Won, P.C. Hoffmann and A. Heller. Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

Reaggregate tissue cultures composed of neurons from the mesencephalic tegmentum and corpus striatum of 14 day old embryonic mice were treated with methamphetamine (meth; 10^{-9} - 10^{-3}M) at 15 days in culture for 7 days. This caused parallel decreases in reaggregate dopamine (DA) levels and cell numbers (visualized by histofluorescence) and reductions in serotonin (5HT) levels. Accumulation of exogenous DA expressed per visualized DA neuron and endogenous GABA levels were not altered by this treatment. Time-course studies showed that the levels of DA are decreased to 38 and 68% of control after 1 day of treatment with 10^{-4}M or 10^{-5}M meth at 15 days in culture and remain depressed after 4 and 7 days of treatment. 10^{-6}M meth decreased DA levels to 63% of control but only after 7 days of treatment. Serotonin levels decreased gradually between 1 and 7 days of treatment with 10^{-4} and 10^{-5}M meth but were not decreased until after 4 days of treatment with 10^{-6}M meth. Thus, it appears that the levels of DA decreased more rapidly than those of 5HT at any given concentration of meth. The potential recovery from effects of meth was assessed by treating reaggregates with 10^{-4}M or 10^{-5}M meth for 2 days, discontinuing exposure to meth and allowing the reaggregates to continue in culture for an additional 5 days. Dopamine and 5HT levels were reduced after 2 days of treatment with meth. However, 5 days after removal of meth, DA levels had recovered while 5HT levels were still decreased. These experiments indicate that the decreases in DA levels can be reversed after removal of meth while the reducation in 5HT levels appears to be more persistent. Supported by MH42134 and NIDA contract #271-87-8114.

507.13

DOPAMINE NEURONS INCREASE THEIR RESISTANCE TO THE NEUROTOXINS MPTP AND MPP^+ DURING DEVELOPMENT IN CULTURE. C. Mytilineou and P. Danias*. Dept. of Neurology, Mount Sinai School of Medicine, New York, N.Y., 10029.

Prerequisites for MPTP neurotoxicity are its metabolic oxidation by monoamine oxidase B (MAO-B) to MPP^+ and the uptake of MPP^+ by the dopamine (DA) neurons. We have studied the relationship between MAO activity and uptake capacity and the efficacy of the neurotoxins MPTP and MPP^+ in dissociated mesencephalic cultures during 4 weeks of in vitro growth. The specific activity of both MAO increased from 18.6±0.6 at 1 week to 68.6 ±3.0 nmoles/mg protein/hr at 4 weeks for MAO-A and from 1.2±0.2 to 24.3±0.6 nmoles/mg protein/hr for MAO-B. The uptake capacity for [^{3}H]DA also increased during development reaching a plateau at 3 weeks. When the effect of MPTP and MPP^+ upon the DA neurons was examined, we observed that, contrary to our expectations, toxicity was diminishing during development. At on week in vitro, exposure to 5 µM MPTP for 4 days resulted in a 77% reduction of [^{3}H]DA uptake sites, while 1 µM MPP^+ during the same time produced a 93.8% reduction. In 2 week old cultures MPTP, under the same conditions, caused a 60.1% and MPP^+ a 52.3% reduction of DA uptake sites. The efficacy of the neurotoxins continued to decrease for the next 2 weeks and at 4 weeks MPTP caused only a 46.3% and MPP^+ 33.5% reduction of uptake sites. Intraneuronal accumultation of [^{3}H]MPP^+ increased during in vitro development. The decrease in toxicity was not associated with an increased storage capacity of the DA neurons. Supported by NIH grant NS-23017 and American Parkison Disease Association.

507.14

ENDOGENOUS MECHANISMS OF PROTECTION AGAINST CATECHOLAMINE TOXICITY IN RAT CEREBRAL CORTEX IN DISSOCIATED CELL CULTURE. S. Vasquez* and P. A. Rosenberg (SPON: S. Fischel). Dept. of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115.

We have previously demonstrated the toxicity of norepinephrine (NE) and other catecholamines to both cortical neurons and glia in dissociated cell culture (J. Neurosci. 8: in press, 1988). This effect occurs at or above 25 uM NE, does not appear to be mediated by adrenergic receptors, and is blocked by catalase. Three types of cultures were used for experiments to be described, all derived from 16 day embryonic embryonic rats: 1) "immature" cultures used at 24 hours in vitro; 2) "mixed-mature" cultures comprised predominantly of astrocytes but containing c. 10% neurons, used at 4-8 weeks in vitro; 3) "neuronal-mature" cultures comprised of 70-90% neurons, used at 2-4 weeks in vitro. Cultures were exposed to NE for 72 hours. In immature cultures, vulnerability to toxicity of 25 uM NE was found to be highly dependent upon culture density. At 250 uM NE this effect of culture density was not demonstrable. In mixed-mature cultures, no evidence of toxicity could be demonstrated even at 250 uM NE. However neuronal-mature cultures were devastated by exposure to 25 uM NE. Further work will attempt to characterize the mechanisms of protection involved as well as their localization, developmental expression, and regulation. This work was supported by a Robert Morison Fellowship from the Grass Foundation, NS 00993, and CH MR Core HD 06276.

MEMBRANE COMPOSITION AND CELL SURFACE MACROMOLECULES III

508.1

THE ROLE OF NCAM IN GM1-MEDIATED NEURITOGENESIS. H.T. Safferstein* and F.J. Roisen (SPON: R. Dagirmanjian). Department of Anatomical Sciences and Neurobiology, School of Medicine, Univ. of Louisville, Louisville, KY 40292.

Exposure of the murine neuroblastoma cell line Neuro-2a to the ganglioside GM1 has been shown to facilitate neuritogenesis. The neurotrophic action of GM1 on Neuro-2a was characterized by assaying ornithine decarboxylase induction and neurite development with light and electron microscopy. Treatment of Neuro-2a with GM1 produced a redistribution of cytoskeletal components. In this study, the role of the neural cell adhesion molecule (NCAM) in GM1-mediated neuritogenesis was examined by determining the distribution of NCAM during several growth conditions. Fluorescent and electron microscopic immunolocalization of Neuro-2a cultures treated with exogenous GM1 revealed a redistribution of NCAM along the perikaryal and neuritic membranes. Neurites produced in response to serum deprivation or dibutyryl cAMP had a different NCAM distribution. Studies are in progress to identify the source of the newly distributed NCAM as well as the cytoskeletal components essential for the resultant redistribution. Supported by NIH grant NS24524.

508.2

ASSESSMENT OF THE BIOLOGICAL ROLE OF THY-1 BY MODIFICATION OF ITS EXPRESSION AND STRUCTURE IN TRANSGENIC MICE. R.J. Morris*[1], C.Mettling*[1], G.Raisman[1], M. Vidal*[2], F. Grosveld*[2] and F.L.Margolis[3], (SPON: P.Kirkwood), Norman & Sadie Lee Res.Ctr., Lab.Neurobiol.[1], & Lab.Gene Structure and Expression[2], Nat. Inst. Med. Res., London NW7 1AA U.K.; and Roche Institute of Molecular Biology, Nutley NJ 07110[3].

Thy-1, a major neuronal surface glycoprotein, is the simplest member of the immunoglobulin superfamily and is therefore thought to be involved in mediating, as yet undefined, cellular interactions in nervous tissue. The molecule appears on virtually all neurons after axonogenesis, with the notable exception of primary olfactory neurons. These cells have the unique ability in mammals to grow normally, and to regenerate, into adult CNS, a property reflected in their unusual interactions with glial cells on entering the olfactory bulb. Will the expression of high levels of Thy-1 on these axons affect these properties? We have constructed hybrid genes containing the Thy-1 protein coding region flanked by the regulatory elements of the olfactory marker protein gene which is uniquely expressed by the olfactory neurons. Transgenic mice have been produced with these constructs, and their pattern of expression, and its functional consequences, will be described. We have also demonstrated (Kollias,G. *et al.* Cell 51:21 1987) that ectopic expression of transgenic Thy-1 in non-neural tissue can cause transformation; we will describe alterations in the Thy-1 structural gene which affect this property.

508.3

CHARACTERIZATION OF ALLELIC FORMS OF 1B236/MAG IN MOUSE. L.H. Farber, C.Lai* and R.J. Milner. Research Institute of Scripps Clinic, La Jolla, CA 92037.

The protein 1B236 was originally identified by analysis of rat brain-specific cDNA clones and has been shown to be identical to myelin-associated glycoprotein (MAG). A single 1B236/MAG gene has been mapped, (D'Eustachio, P., et al., *J. Neurochem.*, 50:589, 1988; C. Blatt, personal communication), near the quivering locus on chromosome 7 of the mouse. Quivering is a recessive mutation in mouse that is characterized by a progressive instability of gait.

We have identified two forms of 1B236/MAG mRNA expressed in different mouse strains using RNase protection experiments. These probably correspond to allelic differences in the 1B236/MAG gene: one form was found in most mouse strains tested, while the second was detected only in DBA/2 and in quivering mice. Because the quivering mutation (*qv*-J) used in these studies arose in a DBA/2 strain mouse, the expression of the DBA/2 allele of 1B236/MAG in quivering mice, but not in the background strain (C3FeB6) for the mutant, suggests that the quivering and 1B236/MAG genes are very closely linked, if not identical.

The mapping data combined with the results of our RNase protection experiments have led us to investigate these allelic differences more thoroughly. We are using nucleic acid sequence analysis to compare 1B236/MAG cDNAs from DBA/2, C3FeB6 and quivering mice. Preliminary data has revealed differences in the sequences of the C-terminal regions. These studies may lend insight into the limits of structural variability allowable in the 1B236/MAG gene product. (Supported by a Fellowship from the National Multiple Sclerosis Society to L.H.F. and grant NS 20728 from NIH).

508.4

1B236/MAG EXPRESSION IN NEURONS C. Lai*, E.L.F. Battenberg, R.J. Milner, F.E. Bloom. Research Institute of Scripps Clinic, La Jolla, CA 92037

The rat protein 1B236 was isolated as a brain-specific gene product and has been shown to be identical to the myelin-associated glycoprotein (MAG). This protein exists in two forms, a longer form whose expression is maximal at approximately postnatal day 25, and a shorter form whose expression peaks at approximately postnatal day 60. We have previously observed 1B236 immunoreactivity in a subset of neurons in both adult and young rats (Bloom, F.E. et al., *J. Neurosci.* 5:1781, 1985 and Lenoir, D. et al., *J. Neurosci.* 6:522, 1986). Here we extend those analyses using antibodies generated against MAG to examine young (postnatal day 21) and adult (>3 months) rats as well as 75 day old mutant *quaking* (*qk*/*qk*) and normal background strain (B6C3Fe) mice. One rabbit polyclonal and two mouse monoclonal antibodies against MAG (gifts of R.H. Quarles, NINCDS) each show a pattern of immunoreactivity similar to that seen with the 1B236 anti-peptide antibodies used in the original surveys. All of these antisera recognize both oligodendrocytes and neurons and reveal a near coincident pattern of expression. In situ hybridization studies were conducted utilizing oligonucleotide probes that distinguish between the mRNAs encoding the two protein forms. Hybridization is observed to mRNA contained within oligodendrocytes and neurons in a pattern consistent with the immunohistochemical results. 1B236/MAG is believed to function as a cell adhesion molecule in oligodendrocytes; whether neuronal 1B236/MAG is involved in similar interactions remains to be established. Supported by NIH grant NS 20728.

508.5

CNS DISTRIBUTION OF MONOCLONAL ANTI-THY 1 ANTIBODY (OX7) AFTER SINGLE INTRAVENTRICULAR INJECTION. T.L. Davis and R.G. Wiley (SPON: P. Loosen) Lab of Experimental Neurology, VAMC, Nashville, TN 37212.

Thy 1 is a surface glycoprotein abundantly displayed on neurons and certain lymphocytes of adult rats. Previous studies have demonstrated neuronal uptake and axonal transport of the monoclonal anti-Thy 1 antibody, OX7, after intraparenchymal or subepineural injection. The present study sought to determine the anatomical fate and neurotoxicity of single intraventricular injections of OX7. 75 - 187 ug of OX7 dissolved in PBS were pressure microinjected (10 - 25 ul) into the left lateral ventricles of anesthetized adult, male Sprague-Dawley rats. After 1-4 d, rats were reanesthetized and transcardially perfused with 4% formaldehyde followed by 30% sucrose in phosphate buffer. 50 um frozen sections of brain and spinal cord were processed to demonstrate mouse IgG using rabbit anti-mouse IgG (Cappel) and biotin-avidin-peroxidase (Vector) with DAB as chromagen. All rats remained healthy after OX7 injections. Immunoperoxidase staining was present: 1 - in the head of the caudate nucleus at the injection site, 2 - superficially along ventricular surfaces, 3 - in the molecular and Purkinje cell layers of the cerebellar cortex, 4 - in the area postrema/nucleus tractus solitarius region of the dorsal medulla, and 5 - within motor neurons of the brainstem and spinal cord. Control rats injected with 200-500 ug of non-immune mouse IgG showed less intense staining than the lowest dose of OX7, particularly in the cerebellar cortex, in spite of the much larger dose. Most staining for OX7 was maximal with 24 hrs survival, although motor neuron staining intensity increased at longer survival times. These results are interpreted as indicating that limited neuronal uptake of OX7 occurs after single intraventricular injections and such injections are not neurotoxic. Chronic infusions and/or higher doses of antibody may be necessary to deliver OX7, or immunotoxins based on OX7, to CNS neurons other than cerebellar cortex. Staining of motor neurons and area postrema may represent systemic distribution of antibody. (This work supported by the Veterans Administration.)

508.6

PURIFICATION OF NEURON-SPECIFIC ANTIGENS FROM ZEBRAFISH. M.B. Bass* and M. Westerfield (SPON: W. Metcalfe). Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Two monoclonal antibodies that recognize antigens on surfaces of specific subsets of neurons in developing zebrafish have been selected for studying mechanisms of neurogenesis.

The Zn-5 antibody labels secondary motoneurons in the spinal cord as well as subsets of cells in the hindbrain. The antigen, recognized by this antibody, has been affinity purified and appears as a band of 75 kDa on a Western blot. The antigen is highly expressed in developing zebrafish, reaching maximal expression at 3 to 4 days after fertilization. Expression then decreases rapidly and by 14 days is extremely low. This temporal pattern of expression correlates with the time these neurons are extending axons and making synapses.

The Zn-12 antibody labels sensory neurons quite specifically in early development but later recognizes nearly all neurons. Affinity purification yields a major band at 65 kDa on a Western blot, although an extremely heterogeneous band is labelled in adult brain extracts with a range of molecular weights from 65 to more than 200 kDa.

Supported by NIH grant HD 22486.

508.7

NCAM REGULATION IN MUSCLE CULTURED IN SERUM-FREE DEFINED MEDIA. J.M. Lyles and C.L. Weill. Dept. of Neurology, LSU Medical Center, New Orleans, LA 70112.

Chemically defined media (CDM) are useful in cellular regulation studies since variations in serum or chick embryo extract (CEE) are eliminated. Two such systems which we developed for chick muscle culture were 5% adsorbed horse serum(aHS)/CDM on collagen matrix and serum-free(SF)-CDM on basement membrane protein matrix. In both systems, cultures developed as large, multi-nucleated, cross-striated myotubes that occassionally contracted. Standard myotube culture media (5%CEE/10%HS/MEM) initially produced straighter, narrower myotubes; after 2 weeks in vitro they appeared similar to SF-CDM cultures. Relative NCAM levels increased 2 to 3 fold during development, from low levels in myoblasts, to peak levels of 400mOD/ug protein (in our ELISA) at myotube day 5-7; subsequently, relative NCAM levels plateaued or decreased slightly due to increasing total protein. NCAM was quantified in primary fibroblast cultures and was present at 10-20% the level in day 5 myotubes. Relative NCAM levels were slightly higher in myotube cultures in 5%aHS/CDM or SF-CDM, as compared to CEE/HS/MEM, probably due to the more rapid disappearance of fibroblasts. The SF-CDM system will be employed to study regulation of NCAM by a variety of biological agents, such as hormones, peptides and other regulatory factors. Supported by NIH grant NS25298-01.

508.8

RPE CELLS ARE LABELLED BY THREE UNIQUE MONOCLONAL ANTIBODIES AND BY ANTIBODY AGAINST LAMININ RECEPTOR. J. M. Neill, J. R. Sparrow, and C. J. Barnstable. Rockefeller Univ., N. Y., N. Y. 10021, and Dept. of Ophthalmol., Yale Univ. Sch. of Med., New Haven, CT 06510.

Retinal pigment epithelial (RPE) cells, derived from the optic cup neuroepithelium, play an important role in metabolite transport, phagocytosis of rod outer segments, and neural retina adhesion. Several monoclonal antibodies directed against RPE (RET-PE3, PE4, and PE5) were derived using a crude rat RPE membrane preparation as the immunogen. Both PE3 and PE4 stained the apical and basal regions of the RPE cell layer most heavily in frozen sections of PN 10 rat eyes, with some specular staining of the cell bodies. The basal region of RPE was stained by PE5, as were vascular structures, indicating binding of a basement membrane (BM) component. Staining patterns on cultured RPE cells showed differences between antibodies: PE3 gave a centrally localized specular pattern at 2 days in culture that was lost after one week in vitro; PE4 stained small, circular, granular bodies adhering to the RPE monolayer at both 2 and 7 days in vitro; and PE5 did not label RPE cultures at either time in vitro. Since adhesion to the extracellular matrix is one way in which RPE cells establish their characteristic polarity, the expression of laminin and laminin receptors was studied in vivo and in vitro. Sections of PN4 and adult rat eyes, stained with either rabbit antisera to laminin or to laminin receptor (gift of L. Liotta), showed codistribution: the receptor localized to the basal RPE surface, while laminin was present in the RPE BM. E16 eyes also showed staining for laminin but not for laminin receptor. RPE cells cultured up to 3 weeks do not label for laminin but do label with laminin receptor antiserum, producing a diffuse, specular central patch of staining. Supported by EY05206.

508.9

FLUORESCENCE-ACTIVATED FLOW CYTOMETRIC ANALYSIS OF MAJOR HISTOCOMPATIBILITY (MHC) CLASS I ANTIGEN EXPRESSION ON NEURAL CELLS FROM THE DEVELOPING HUMAN NERVOUS SYSTEM. B. A. Boyer* and B. Wigdahl (SPON: R. J. Ziegler). Department of Microbiology, The Pennsylvania State Univ. Col. of Med., Hershey, PA 17033.

The MHC class I antigens expressed on most nucleated cells in humans are essential for reactions of immune recognition. In particular, MHC class I antigens in conjunction with viral antigen are recognized by human cytotoxic T lymphocytes, resulting in destruction of virus-infected cells. Previous studies with central nervous system (CNS) material have demonstrated low levels of antigens encoded by the MHC. As previously observed in the development of many human tissues, MHC class I expression has been shown to be more prevalent in adult CNS tissue. Current studies are investigating the expression of MHC class I antigens in the developing human peripheral nervous system utilizing neural cell populations obtained by enzymatic dissociation of the dorsal root ganglia with associated peripheral nerves after removal from aborted human fetal specimens. Fluorescence-activated flow cytometric analysis was performed on live human fetal neural cell populations using a primary monoclonal antibody specific for the heavy chain of the HLA-ABC. MHC class I antigen expression was examined between the gestational ages of 12 and 16 weeks. These studies have indicated that the number of HLA-ABC antigen-positive cells varied between 59 and 87% in tissue isolated between 12 and 16 weeks gestation. In parallel studies, we examined the fetal DRG cell population using the A2B5 monoclonal antibody which reacts primarily with the GQ ganglioside of neurons in fetal DRG. These studies have demonstrated a 20-30% reduction in the number of A2B5-positive cells between 12 and 16 weeks gestation. The differential expression of MHC antigens on the surface of glial and neuronal cells in the developing human nervous system may be important in determining susceptibility to virus infection.

508.10

IMMUNOREACTIVITY TO ANTI-SYNAPTIC VESICLE PROTEIN IN THE RETINA AND PARIETAL EYE OF A LIZARD. G. A. Engbretson and M. A. Ringwood*. Institute for Sensory Research, Syracuse University, Syracuse, New York 13244.

The parietal eye and retina are two distinct vertebrate photoreceptive organs which display some structural and functional commonalities.

Retinal and parietal eye tissues were examined for immunoreactivity to anti-synaptic vesicle protein antibodies†. The antibodies label a 36 kDa protein (SVP-36) from the synaptosomal fraction of guinea pig cerebrum and they have been seen to label synaptically associated structures in several vertebrate classes.

In the retina the immunoreactivity was heavy in the inner plexiform layer (IPL) with distinctive banding in sublayers 2, 4, and 5. Labeling was also found surrounding most of the cells near the IPL-inner nuclear layer boundary, probably amacrine cells. Very light labeling was found in the outer plexiform layer (OPL) and was mostly restricted to a thin layer where the OPL and the horizontal cells meet. Some immunoreactivity was localized in patches in the optic nerve fiber layer. In the parietal eye the immunoreactivity was similar in density to the retinal OPL labeling though not layered in any discernable pattern.

The immunoreactivity to anti-SVP-36 is to be expected in the IPL of the retina. Electron micrography showed a high density of synaptic junctions there. However, the greatest density of synaptic vesicles in both the retina and parietal eye was seen in the photopreceptor synaptic terminals, where anti-SVP-36 immunoreactivity is sparse. Evidently synaptic vesicles from photoreceptors differ in composition from those in neurons of the IPL.

†We thank Dr. K. Obata for his generous gift of antibodies.

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508.11

CONNEXIN$_{43}$ IN BRAIN AND BRAIN CELL CULTURES. R. Kadle*, R.E. Fellows[1] and B.J. Nicholson*. (SPON: M.S. Hudecki) Dept. of Biol. Sci., SUNY, Buffalo, NY 14260 and [1]Dept. of Physiology & Biophysics, Univ. of Iowa, Iowa City, IA 52242.

Multiple variants of gap junction proteins called Connexins (Cx) have been demonstrated in several tissues. The variants include Cx$_{32}$ and Cx$_{26}$ (the subscript referring to molecular weight in kD) first detected in liver, and Cx$_{43}$ first detected in heart. Previous studies from our laboratory as well as others have demonstrated the presence of Cx$_{32}$ and Cx$_{26}$ in adult rat brain, specifically in cerebellum. The present study was carried out to investigate the presence of Cx$_{43}$ in adult rat brain and in cells cultured from fetal rat brain. Neuronal and astroglial cultures prepared from 18-day fetal telencephalon and newborn cerebral cortex respectively were maintained in a serum-free defined medium. A polyclonal anti-peptide antibody to the first 20 N-terminal amino acids of Cx$_{43}$ was affinity purified using the synthetic peptide. SDS PAGE and western blotting of homogenates indicated the presence of a 38 and 40 kD proteins in brain homogenates compared to 38 and 43 kD proteins in heart homogenates. Although both 38 and 40 kD bands were observed in astroglial and neuronal cultures, the latter was markedly more prominent in glial cultures. Degradation products of identical MW were seen in both heart and brain homogenates. These results indicate the presence of proteins related to Cx$_{43}$ in adult and fetal brain and suggest that gap junctions may play a role in the development and regulation of CNS function. Supported by NIH grants HL37109-03 (BJN) and ROI NS24629-02 (REF).

508.12

REGULATION OF SCHWANN CELL SURFACE NERVE GROWTH FACTOR (NGF) RECEPTORS BY NEURONAL MEMBRANES. P.S. DiStefano and D.M. Chelsea*. Neuroscience Research Division, Pharmaceutical Discovery, Dept. 47W, Abbott Laboratories, Abbott Park, IL 60064.

Schwann cells isolated from rat sciatic nerve express high levels of NGF receptors when maintained in primary tissue culture. Experiments were carried out to examine the ability of neurons or neuronal membranes to regulate Schwann cell NGF receptor number when co-cultured with Schwann cells. Mouse superior cervical ganglion (SCG) neurons were co-cultured with rat Schwann cells for 1 wk. Schwann cells were assayed for NGF receptors using a 2-site immunoassay specific for rat NGF receptors. Mouse SCG neurons induced Schwann cell proliferation followed by down-regulation of Schwann cell NGF receptor binding. The degree of receptor down-regulation was dependent on the number of neurons co-cultured with Schwann cells. Crude membranes prepared from postnatal day 7 (P7) mouse sciatic nerve or spinal cord also caused proliferation and down-regulation of Schwann cell NGF binding after 3 days of co-culture. Membranes from P7 cerebellum, cerebral cortex, or liver had no receptor regulating activity. Membranes from P0, P14 and adult mouse spinal cord were also effective in regulating Schwann cell NGF receptor number. Preliminary experiments showed that receptor regulating activity was solubilized from spinal cord membranes with Triton-X-100 and that upon boiling the extracts activity was lost. These results show that an NGF receptor regulating activity is found in axon-rich preparations from the both the central and peripheral nervous systems.

508.13

MACROMOLECULAR STRUCTURE OF AXON MEMBRANE FROM md AND md HETEROZYGOTE OPTIC NERVES. J.A. Black, I.D. Duncan[1], S.G. Waxman, B.R. Ransom and K.F. Jackson*[1]. Dept. of Neurology Yale Univ. Sch. Med. and V.A. Med. Ctr., West Haven, CT 06516, and [1]Dept. of Medical Sci., Sch. Vet. Med., Univ. Wisconsin, Madison, WI 53706.

Myelin deficient (md) rat is a sex-linked trait which causes hypomyelination within the CNS. Female heterozygotes (mdH) exhibit CNS myelin mosaicism. As part of a study to examine the influence of glia on axon membrane structure, axolemma from md (25 d) and mdH (15 mo) optic nerve (ON) was investigated with freeze-fracture EM.

Mean$\pm$SD intramembranous particle densities for md and mdH amyelinated and mdH myelinated axons are:

Condition	PF	EF
md - pooled amyelin.	1549$\pm$572.0	177$\pm$84.3
- <.4 um dia.	774$\pm$133.4	172$\pm$22.5
- .4-.59 um dia.	1563$\pm$464.5	190$\pm$104.7
- >.6 um dia.	1886$\pm$710.1	144$\pm$32.2
mdH - pooled amyelin.	1290$\pm$518.0	155$\pm$68.5
- <.4 um dia.	753$\pm$355.7	168$\pm$43.1
- .4-.59 dia.	1459$\pm$374.9	161$\pm$72.5
- >.6 um dia.	1603$\pm$432.1	140$\pm$78.9
mdH - internode	1755$\pm$557.4	158$\pm$36.6

These data indicate that amyelinated axon membrane from md and mdH ON display similar ultrastructure, and that larger diameter (>.4 um) md and mdH amyelinated axons exhibit membrane structure similar to internodal membrane. Supported in part by grants from NIH, NMSS and VA

509.1

HIGH AFFINITY CHOLINE UPTAKE OF HIPPOCAMPAL SYNAPTOSOMES IN RESPONSE TO ENDURANCE TRAINING IN YOUNG AND OLD RATS

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Aging has been associated with a progressive decline in hippocampal cholinergic function. Endurance exercise has been shown to modify characteristics of brain neurons and their receptors. Due to the involvement of the hippocampus in initiation and control of voluntary movement as well as memory, it was of interest to determine whether endurance exercise would alter acetylcholine metabolism of the hippocampus in young and old animals. High affinity choline uptake, the rate limiting step in acetylcholine synthesis, was determined in synaptosomes of the hippocampus of endurance trained rats and their age matched sedentary controls. Male F344 rats were run on a treadmill for 6 months for 30 min./day at a speed of 20 m/min. Young rats, originally at 6 months of age, and old rats, originally at 19 months of age were killed by decapitation at 12 months and 25 months of age, respectively, and the synaptosomes of the hippocampus isolated. The high affinity choline uptake was determined by incubating the synaptosomes with 0.75µM (1.92µCi) 3H-Choline in both a sodium containing and sodium-free medium. Comparison of synaptosomes of untrained, young and old rats showed a 35% ($p<.05$) reduction in high affinity choline uptake which is consistent with previous reports of an age related reduction of cholinergic function. The synaptosomes of young trained rats demonstrated a 29% ($p<.02$) reduction (15.3 pmoles/mg vs. 10.8 pmoles/mg) in high affinity choline uptake. This reduction indicates alterations in presynaptic cholinergic function which may influence cholinergic synaptic transmissions and/or acetylcholine turnover. Old trained rats showed no significant difference from their age matched controls indicating a loss of synaptic adaptability in aged animals.

509.2

ENDURANCE EXERCISE ALTERS NMJ MORPHOMETRY IN C57BL/NNia MICE GLUTEUS MAXIMUS MUSCLE DURING AGING.

M.H. Andonian, D. Weese And M.A. Fahim. USC Andrus Gerontology Center, Los Angeles, CA 90089-0191.

Previous studies indicate that exercise modulates age-related changes in the neuromuscular junction, and that tonic muscles are less affected by endurance training (Andonian & Fahim, J. Neurocyt. 16, 589-99, 1987). To further study these phenomena, the effects of age and endurance exercise on the morphology of ZIO-stained gluteus maximus NMJs from 12, 18, 24 month old C57BL/NNia mice were assessed under identical conditions. This muscle is recruited to maintain posture and for locomotion. It is predominantly slow-twitch, but is a mixed muscle in the mouse.

Animals were exercised at 26-28 m/min for 60 min/day 5 days/wk for eight weeks. Camera lucida drawings made from ZIO-stained NMJs of control and exercised gluteus maximus fibers from 12, 18, 24 months were measured using a computer-aided morphometry package. Repeated-measures multivariate analysis of covariance was used to test for differences in nerve terminal area, perimeter, and extent length between ages and test conditions.

There were no significant changes in nerve terminal area between 12, 18, and 24 months of age, although qualitatively there was an upward shift in distribution of the morphometric parameters with age. Nerve terminal perimeters and lengths were significantly larger at 24 months than 12 and 18 months, with 18 month values being smaller than 12 month data. This is similar to age-related elaboration observed in slow-twitch muscle, but is of lower magnitude than fast twitch muscle. At 12 months, exercised nerve terminals were larger than controls, while at 18 months they were smaller. This data is similar to that previously observed in the soleus and EDL. However, the areas and lengths of the exercised 24 month animals were larger than controls, although the perimeters did not change. These data indicate that exercise does modulate the morphology of the NMJ, however the magnitude and direction are dependent on age, fiber type and other factors.

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509.3

AGE-RELATED CHANGES IN α_1-ADRENERGIC RECEPTOR DENSITY IN RAT BRAIN: SCATCHARD ANALYSIS USING QUANTITATIVE AUTORADIOGRAGHY.

D.M. BURNETT and N.R. Zahniser Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

It is well documented that the electrophysiological responsiveness of the CNS to noradrenergic stimulation is diminished with aging. To determine whether receptor changes accompany this diminished responsiveness, we have studied the effects of aging on the density of α_1-adrenergic receptors (AR's) in circumscribed areas of the rat brain using computer-assisted quantitative autoradiography. Sagittal sections of Fischer 344 rat brain were prepared from three aged groups: 4-5, 16-18 and 24-28 months. Saturation curves were generated using 1-200 pM [^{125}I]BE-2254 (IBE) , an α_1-selective antagonist. Nonspecific binding was defined with 1 µM prazosin. Specific IBE binding in discrete brain areas was normalized by measuring corresponding protein levels with a densitometric imaging assay. Scatchard analysis revealed signifigant age-related decreases in the density of α_1-AR's in the thalamus and olfactory tubercle, but not in cortex, striatum, hippocampus, cerebellum or brain stem. In the thalamus Bmax= 1067 ±88 fmol/mg protein at 4 months of age vs 771 ±118 fmol/mg protein at 24-28 months. In the olfactory tubercle, corresponding values were 425 ±38 vs 249 ±48. No affinity changes were found. Preliminary data suggest that signifigant decreases in the number of α_1-AR's are also evident by 16-18 months of age. These studies found that with aging, decreases in the number of central α_1-AR's are not ubiquitous but are confined to specific areas. Supported by USPHS AG-04418 and the PMA Foundation.

509.4

CYTOSOLIC CALCIUM CONCENTRATIONS IN CORTICAL SYNAPTOSOMES OF AGING RATS.

L. Giovannelli and G. Pepeu*. Dept. of Pharmacology, Florence University, 50134 Florence, Italy.

Cytosolic calcium concentrations were measured in purified synaptosomes from the cerebral cortex of 3,16 and 24 month old male Charles River Wistar rats, by the Quin-2 technique. Electron microscopy examination of the synaptosomes revealed a 15% contamination by mitochondria. Synaptosome samples were incubated at 37 °C in oxygenated medium, pH 7.4, and loaded with 20-30 µM Quin-2AM for 15 min. After dilution, centrifugation and resuspension of the samples, readings were made in a Perkin Elmer spectrofluorimeter. Neither calcium concentration at rest (124±6 nM) nor the increase after potassium (50 mM) depolarization was modified by age. The calcium load following depolarization was cleared in about 13 min in 3 month old rats. The rate of clearance was significantly slower in 24 month old rats. The addition of verapamil (60 uM) or nimodipin (10 uM) after depolarization restored calcium concentration to resting level in aged at the same rate as in young rats. An increase in calcium influx mediated through L channels may therefore be responsible for the slower clearance of calcium load in aged rats. Work supported by a CNR grant.

509.5

EXTREME SHIFTS IN CHOLINE UPTAKE AND ACETYLCHOLINE TURNOVER CONSISTENT WITH INTEGRITY OF PHYSIOLOGIC REGULATORY MECHANISMS DURING AGING.

D.O. Smith and M.R. Chapman*. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

Uptake of labeled choline and its incorporation into ACh were assayed at the neuromuscular junction of the extensor digitorum longus muscle of rats aged 11 and 27 months. Under resting conditions, ACh levels per nerve terminal were 72% higher in the aged animals. However, the specific activities of ACh and choline were nearly 10-fold higher in the 11-month animals, indicating negligible uptake of labeled choline in the older animals. During nerve stimulation (1 Hz), the amount of labeled choline incorporated into ACh was 150% greater in the aged animals, although total ACh content decreased by about 60%. The specific activity of ACh released during stimulation was significantly greater in the 27-month animals, but the amount released did not change appreciably with age. These results suggest that elevated ACh levels in the aged animals (due to much reduced quantal release) inhibit choline uptake under resting conditions. During nerve stimulation, significantly more choline from extracellular sources thus enters the ACh synthesis pool in the older animals. These shifts are consistent with intact physiologic regulatory mechanisms during aging. Supported by NIH grant AG01572.

509.6

AGING AND MUSCARINIC RECEPTOR SUBTYPES: AUTORADIOGRAPHIC ANALYSIS IN RAT AND HUMAN BRAIN.

A. Biegon, B. Earley*, M. Hanau*, and M. Segal; Weizmann Inst. Sci., Rehovot, Isr.

As a step towards the characterization of age effects on the cholinergic system, we compared the distribution of muscarinic M1 and M2 receptors in brains from young and old subjects, using quantitative in vitro receptor autoradiography. Twenty four drug and pathology free human brains (age range 17 to 81 years) were collected at autopsy from the medicolegal institutes in New York and Jaffa. Seven young (4 months) and 6 old (28-31 months) rats were also used. Brains were frozen in powdered dry ice. M1 and M2 receptors were labeled on coronal, 30 or 40µ cryostat sections using 1nM 3H-QNB (N.E.N., s.a.24.3Ci/mmole) in the presence of 100µM carbachol or 200nM pirenzepine respectively. Non specific binding was assessed in the presence of 1µM atropin. The autoradiograms were analyzed by an IBM PC based computerized image analysis system. A pattern of anatomically selective, age related decreases in M1 and M2 receptors was observed in both human and rat. M1 receptors were significantly decreased (10-30%) in cortex striatum and several subregions of the hippocampus. M2 receptors in cortex and striatum were decreased, while hippocampal receptors were not. A large decrease (more than 50%) in M2 receptors was observed in the substantia innominata and other ventral forebrain cholinergic nuclei. The location and size of the age-related changes were similar in human and rat.

509.7

Age-Related Electrophysiological Changes in Cerebellar Noradrenergic Receptors. K.D. Parfitt*, R. Freedman and P. Bickford-Wimer (SPON: L. Adler). Depts. of Pharm. & Psych., Univ. of CO Health Sci. Ctr. and VAMC, Denver, CO 80262.

Previous investigations have shown deficiencies in noradrenergic transmission in the central nervous system of aged laboratory animals. In particular, subsensitivity to locally-applied norepinephrine has been observed when recording extracellularly from cerebellar Purkinje neurons of aged rats. In young rats alpha$_1$, alpha$_2$ and beta adrenergic receptors are present and functional in the cerebellar cortex. It was the purpose of this study to determine which, if any, of these receptor subtypes show altered responses with age. The ability of selective noradrenergic agonists to inhibit the spontaneous activity of Purkinje neurons was compared in young (3-mo.) and aged (18- and 28-mo.) F344 rats. Drugs were applied to Purkinje cells by pressure micro-ejection from multibarreled micropipettes and a paired-pipette paradigm was used to compare the potency of each pipette in young and aged rats. Purkinje cells of young rats were significantly more sensitive to locally-applied isoproterenol, a beta-adrenergic agonist, than Purkinje cells of both of the older age groups. Subsensitivity to the alpha$_1$ agonist phenylephrine and the alpha$_2$ agonist clonidine was not observed in the aged rats. These results suggest that postsynaptic sensitivity to beta-adrenergic agonists decreases with senescence, whereas postsynaptic sensitivity to alpha$_1$ and alpha$_2$ agonists does not change. Work supported by USPHS Grant AGO4418 and the VAMRS.

509.8

NEUROMOTOR COORDINATION MECHANISMS AND AGING. N.C. Rich. PHS Dept., Miami Univ., Oxford, OH 45056

The purpose of this investigation was to examine neuromotor coordination mechanisms in young and old males during ballistic forearm flexion and extension tasks. The subject sample included 16 males in each of the following groups: (1) 30-40 years old, (2) 50-60 years old, and (3) 61-70 years old. The IEMG patterns of the biceps brachii (agonist) and triceps brachii (antagonist) muscles were recorded during unresisted and resisted trials. Sixteen temporal and ten quantitative parameters were examined to evaluate between group differences. The parameters which exhibited significant differences between groups were: (1) agonist first burst silent period, and, (2) accuracy. In addition, age-related trends were found to exist for the following parameters: (1) movement time, (2) agonist and antagonist first burst motor time, (3) antagonist second burst duration, (4) agonist first burst IEMG slope, (5) antagonist second burst time to peak activity, (6) time to maximum acceleration, (7) agonist first burst and antagonist second burst peak amplitude, and (8) acceleration as a percentage of movement time. For forearm flexion strength groups 2 and 3 were 74.15% and 74.15% as strong as group 1, respectively. For forearm extension strength, groups 2 and 3 were 89.12% and 83.90% as strong as group 1, respectively. The data indicate that while loss of strength is indeed a true concomitant of the aging process, an individual's muscle patterns remain relatively stable into their seventh decade.

509.9

DIFFERENTIAL INFLUENCE OF THE MEDIAL SEPTUM ON CA1 "PLACE" CELLS AS A FUNCTION OF AGE. S.J.Y. Mizumori, C.A. Barnes, and B.L. McNaughton, Dept. Psychology, Univ. Colorado, Boulder, Colorado 80309.

To examine the effects of aging on septal modulation of the behavioral correlates of different hippocampal cell types, 4 young (11 mo) and 4 old (25 mo) Fischer-344 rats were trained to perform a working memory task on an 8-arm radial maze. Dorsal hippocampal neurons were recorded using stereotrodes (McNaughton, et al., J. Neurosci. Meth.,1983) mounted on moveable microdrives. A guide cannula was also implanted to permit local application of 2% tetracaine into the medial septum. When hippocampal units were encountered, the unit-behavior correlate was characterized during the first 5 trials of a recording session. The septum was then reversibly inactivated via tetracaine injection (0.5 µl). Post-injection behavioral performance and unit activity were monitored for an additional 10 trials.

Following septal inactivation, place-specific firing was maintained in 91 % of the CA1 "place" cells (N=22) from young rats, in spite of severely impaired performance on the maze and a dramatic reduction in activity of 64% of dentate gyrus cells (N=50), which include hilar "place" cells, basket and granule cells (also reported by Mizumori et al., Soc. Neurosci. Abst., 1987). In contrast, only 25% of CA1 "place fields" (N=24) from old rats were maintained during the period of behavioral impairment. Similar to young rats, 75% of dentate cells from old rats (N=57) showed reduced firing after tetracaine injection. These findings suggest at least the following two possibilities: 1) the inputs to CA1 "place" cells from within the hippocampus are more highly distributed in young rats than in old rats, and/or 2) direct inputs from entorhinal cortex to CA1 maintain place fields in young, but not old rats.

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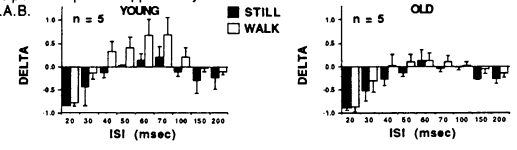
509.10

AGE - RELATED DECREASE IN PERFORANT PATH EVOKED SPIKE FACILITATION IN RAT FASCIA DENTATA E.J. Green, C.A. Barnes and B.L. McNaughton, Department of Psychology, University of Colorado, Boulder, CO 80309.

Prestimulation of the perforant path (pp) at short intervals inhibits subsequent pp-evoked granule cell discharge (spike inhibition) by activating feedforward and feedback inhibitory processes. At longer intervals such prestimulation produces a net increase in granule cell excitability (spike "facilitation"), the mechanism of which is not well understood. Spike inhibition and facilitation appear to result from the interaction of two distinct (inhibitory and facilitatory) processes which can vary independently of one another (Green et. al.,Soc. Neurosci. Abstr.,1987). Furthermore, animals walking on a treadmill exhibit robust increases in spike facilitation relative to when they are still; these differences are not a consequence of granule cell discharge, or of changes in the amplitude of the EPSP. Walking does not appear to affect inhibition elicited by pp stimulation, but does have a substantial influence on the late increase in granule cell excitability which follows pp stimulation (Green et. al., ibid).

Paired-pulse inhibition and facilitation were evaluated over a range of inter-stimulus intervals (20-200 msec) in young (10-14 month) and old (22-26 month) rats walking on a treadmill or sitting quietly in the same apparatus. Young and old rats exhibited comparable levels of spike inhibition at very short ISIs. At longer ISIs, however, old rats showed significantly less spike facilitation than young rats. These results suggest that there is an age-related decline in a process which acts to increase granule cell excitability following activation of the perforant path. Supported by F32 AG05345 to E.J.G. and AG03376 to C.A.B.



509.11

DIETARY ACETYL-L-CARNITINE IMPROVES SPATIAL BEHAVIOR OF OLD F-344 RATS. C.A. Barnes, A.L. Markowska*, C. J. Elkins*, J. Kaufman*, & D. Olton. Univ. Colorado, Boulder, CO, 80309, & Johns Hopkins Univ., Baltimore, MD 21218.

Acetyl-L-Carnitine (AC), a natural component of mammalian tissue, plays an essential regulatory role in fatty acid oxidation (Bremmer, 1977). While the highest levels of AC in the body are found in muscle and heart, a metabolic pool of carnitine also exists in the central nervous system (Shug et al., 1982). A number of pharmacological properties of AC suggest its possible efficacy in attenuating age-related neural deficits. These include the observations that AC has cortical cholinomimetic properties (Falchetto et al., 1971), it increases cerebral glucose utilization (Fariello et al., 1986) and free radical scavengers (Ferraro et al., 1986). Furthermore, chronic treatment with AC slows the occurrence of morphological changes that normally occur in hippocampus of old rats (Angelucci et al., 1986), and improves spatial orientation in a circular water pool task (Ghirardi et al., 1986).

The present experiment was designed to replicate, and to extend the observations regarding the attenuation of spatial memory deficits in old rats. Thirty F-344 rats were obtained at 16 mo of age in each of two separate laboratories. Half of the animals received 75 mg/kg AC in their drinking water for six months prior to the initiation of behavioral testing on the Barnes circular platform task (8 young control animals were also included in one laboratory). In this task the animals must learn the spatial location of a hidden escape tunnel that remains fixed beneath one of 18 holes on the periphery of the platform surface. Animals were given one trial per day for 16 days with the goal in one position. The young control and old AC animals made statistically fewer errors by the end of training than did the old control rats: On day 17 the goal was changed to a different spatial location. Both the old AC group and the young controls made significantly more errors than the old control animals in the initial reversal trial, indicating better retention of the original correct location. When the ratio was taken of asymptotic performance on the regular trials to the first reversal trial, a significant main effect of Groups was found ($p < .006$). The old AC animals were significantly different from the old controls, but not from the young controls. These data suggest that dietary AC may be effective in attenuating certain cognitive impairments occurring during the process of normal aging. Supported by Sigma Tau Corp.

509.12

A LIFESPAN STUDY OF HIPPOCAMPAL NEURONAL RESPONSIVENESS TO IONTOPHORETIC APPLICATION OF *L*-GLUTAMATE *IN VITRO*. G. Rao, C. A. Barnes, B. L. McNaughton, and C. Stengel*. Department of Psychology, University of Colorado, Boulder, CO 80309, and Brainwave Systems Corp., Broomfield, CO 80020.

Barnes and McNaughton (J. Physiol. 309: 473, 1980) observed an increase with senescence of the ratio of EPSP to presynaptic fiber potential in granule cells of the fascia dentata *in vitro*, suggesting that in spite of the overall loss of afferent fibers, perforant path synapses were on average slightly stronger in old animals. In addition, an increase in the ratio of population spike to synaptic response was seen in the older animals, suggesting an increased excitability of the postsynaptic neurons. As a continuation of these studies, we have investigated the effects of aging on extracellularly recorded single unit discharge following iontophoretic application of *l*-glutamate in the three principal hippocampal subfields *in vitro*.

A total of 354 FD, 307 CA1, and 298 CA3 cells were studied from 30 animals with ages distributed uniformly between 7 and 26 months. Single units were recorded with glass-filled pipettes (7-10 micron tip diameter) filled with 0.2 M *l*-glutamic acid in calcium-free Ringer's solution. Glutamate application consisted of ten 10 second negative current pulses (7.4 nA) separated by 15 seconds each. Average firing rates were computed during the 10 seconds prior to the glutamate applications and the 10 seconds during them. Glutamate effects were assessed by computing the difference in firing rate between glutamate application and control conditions divided by their sum. Glutamate application produced a substantial increase in firing rate in all three areas. However, there was no significant linear regression on age of either the spontaneous background activity or the relative elevation of firing rate during glutamate activation.

We conclude that aging does not lead to any substantial alteration in the sensitivity of hippocampal neurons to exogenously applied glutamate.

Supported by grant AG03376 to C.A.B.

509.13

AGING ELIMINATES THE DIURNAL RHYTHM AND DEPRESSES THE DENSITY OF ALPHA-1 RECEPTORS IN SELECTED BRAIN AREAS. N.G. Weiland and P.M. Wise. Dept. Physiology, Univ. of Maryland, School of Medicine, Baltimore, MD 21201.

The densities of α_1-adrenergic receptors exhibit a diurnal rhythm in selected hypothalamic nuclei of ovariectomized (OVX) young rats. In intact rats at 1000h α_1 receptor densities increase or decrease with age depending on the brain region and/or reproductive status. We wished to determine whether the age-related changes in α_1 receptor densities result from shifts in the diurnal rhythm and/or occur independently of reproductive status. Utilizing autoradiographic procedures, we measured the density of α_1 receptors in young, middle-aged, and old OVX rats at various times of day. In the medial preoptic nucleus, the diurnal rhythm of the density of α_1 receptors was abolished in middle-aged rats, and receptor densities were decreased in old rats. In suprachiasmatic nucleus and ventral medial nucleus, α_1 receptor densities were suppressed in middle-aged rats, and a further decrease occurred in old animals. In median eminence α_1 receptor densities declined progressively and were significantly decreased in old rats. No age or time-associated changes in α_1 receptor densities occurred in lateral septum. The data demonstrate that during middle-age there is an initial loss of the diurnal rhythm in α_1 receptor densities followed by a progressive decline in α_1 receptor concentrations in older animals. AG-05357, AG-02224.

509.14

CHANGES OF N TYPE CALCIUM CHANNELS DURING AGING R.M.Moresco*,S.Govoni*,O.Gandolfi+,F.Rossi*^, F.Battaini#, M.Trabucchi#.*. Dept. of Pharmacobiol., Univ. of Bari, +Inst.of Pharmacol. Univ. of Bologna,^Sci. Inst. Sanatrix,Venafro, #Chair of Toxicol. II Univ. of Rome, ITALY.

An age-related decrease in stimulated calcium uptake has been described in various brain areas (Gibson, Neurobiology of Aging, 8:328,1987).This observation suggests the existence of an altered functioning of potential-dependent calcium channels. Along this line we previously observed an aged-dependent decrease of dihydropyridine binding sites affinity and sensitivity to calcium (Brain Res.333:374,1985). The present work studies whether N type potential-dependent calcium channels labelled with (125)I-omega-CTx are modified in membranes prepared from cortex and hippocampus of old rats. In the cortex of aged rats we found a reduced omega-CTx binding (90.8±9.1 and 58.3±5.5* ($p<0.05$) f.moles/mg prot. for 3 and 24 month old rats respectively, using 10pM (125)I-omega-CTx). No significant changes were observed in hippocampus indicating an area selectivity of N channel impairment. On the other hand the observed changes in cortex may participate to the impairment of calcium uptake and neurotransmitter release observed in this brain area in aged rats.

509.15

THE EFFECTS OF AGING AND ETHANOL ON NEURONAL ELECTRIC MEMBRANE PROPERTIES. T.A. Bunting*, B.S. Scott. Zoology Dept. U of T, Surrey Place Centre, Toronto, ON. M5S-2C2, Canada.

Intracellular recordings were made from 825 freshly dissociated DRG neurons of young (12-16 wk), mid-aged (48-52 wk) and aged (101-105 wk) mice (C57BL/6NNIA2) in 3 ethanol concentrations [0.0%, 0.5% and 1.0% (v/v)]. A variety of electric membrane properties were determined quantitatively: Specific membrane resistance (RM), capacitance (CM), time constant (T), depolarization threshold (VRH) for action potential (AP) generation, AP duration (DT), AP overshoot (OS), rate of decay of afterhyperpolarization (AHP2), AP rise (RISE) and falling phases (FALL1 and FALL2 for APs with biphasic falling phases). Total neuronal surface area was also calculated. A two way ANOVA indicated no significant interactions between ethanol and age effects. Therefore, the results were pooled and % differences were calculated for control and experimental conditions. Significant ($p<.01$) differences between zero ethanol (n=329) and 1% (n=271) ethanol conditions included a 45.8% increase in VRH (decreased electrical excitability), 22.7% decrease in CM, 12.8% decrease in T and an 11.3% increase in RM. Also, DT decreased 3.7% but only at $p=.052$. Significant differences ($p<.001$) between the young (n=297) and aged (n=271) neurons included a 60.2% increase in AHP2 a 52.1% increase in OS, 27.6% increase in VRH and a 30.9% increase in DT (due to decreased RISE, FALL1 AND FALL2). RM increased 11%, CM decreased 11% and, in addition, a small (4.56%) decrease in surface area was noted. Both ethanol and aging caused a decrease in electrical excitability and capacitance and an increase in membrane resistance. However, whereas age increased AP duration, ethanol decreased DT. The direction of the age effects are similar to those observed using cell cultures (Scott and Lew, in press, 1988). Increased AHP duration was also reported in hippocampal slices in dentate granule cells (Landfield and Pitler, Science, 226, p. 1089, 1984). These findings suggest that aging may alter EMP in DRG in situ by prolonging the AP and AHP and decreasing electrical excitability. Supported by NSERC and OMHF.

509.16

ELECTROPHYSIOLOGICAL STUDY OF THE SUBSTANTIA NIGRA PARS COMPACTA NEURONS IN YOUNG AND AGED WISTAR RATS. PRELIMINARY RESULTS. M.A. Lavín and R. Drucker-Colín. Instituto de Fisiología Celular, UNAM, México.

The substantia nigra pars compacta (SNC) cells are involved in motor control. There is evidence indicating that some characteristics of SNC cells are altered in aged rats (McGeer, E. and McGeer, L. Ergot compound and Brain function. Ed. by Goldstein, 1980).

Male Wistar rats young (3 months old) and aged (20 month old) were anesthetized with halothane, tracheostomized and fixed to a stereotaxic apparatus. Extracellular activity was recorded using single glass capillaries filled with 2% pontamine blue in 0.5 M sodium acetate (8-16 Mohms). Tip positions were marked by ejecting the dye at the end of the experiment.

15 Cells was recorded in young rats with a frequency rate of 40.5 ± 23.4 spikes/10 sec. ($\bar{X}$ ± S.D.) and 7 cells in aged rats with a frequency rate of 31.2 ± 11.6 spikes/10 sec. Although there is not difference in the average frequency rate, there is a difference in the pattern of discharge. In aged rats almost all cells (71%) have a tendency to discharge in bursts of 3 spikes, sometimes alterned with a single spike. In young rats, 73% of the cells have a tendency to discharge in a single spike. This analysis and more recording of SNC cells are in progress.

509.17

A CORTICOSTEROID-SENSITIVE COMPONENT OF THE HIPPOCAMPAL CALCIUM-DEPENDENT AFTER-HYPERPOLARIZATION INCREASES WITH AGING. D.S. Kerr* and P.W. Landfield, Dept. of Phys. & Pharm., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

Although the hippocampus is a major target structure for corticosterone (CORT), little is known about its neuronal effects (*cf.* McEwen, 1982). In addition to its presumed normal actions, CORT also appears to increase the rate of aging changes in the rat hippocampus (Landfield *et al.* 1981; Sapolsky *et al.* 1985). One clue to the effects of CORT may lie in findings that Ca-dependent processes are increased in aged hippocampus (Landfield, Pitler, *Science* 1984; *J. Neurophysiol.* 1986).

In the present studies, therefore, we examined a defined Ca-dependent membrane conductance, the Ca-dependent, K-mediated afterhyperpolarization ($AHP_{(Ca)}$) in young (4-7 mo.) and aged (24-27 mo.) adrenalectomized (ADX) and intact rats. In intracellular recordings from CA1 cells in hippocampal slices the $AHP_{(Ca)}$ was substantially reduced in ADX rats, in comparison to intact rats. This effect of ADX was more pronounced in the aged rats, and the previously-observed age-related increase in AHP duration (Landfield, Pitler, *ibid.* 1984) was therefore abolished by ADX. The AHP could be lengthened in ADX rats treated with injections of CORT prior to study.

These results suggest that an identified Ca-dependent membrane conductance is partially CORT-dependent and that the impact of CORT on this conductance increases dramatically with aging. Because excessive Ca influx has been implicated in cell death, these findings raise the possibility that increased CORT-dependent Ca influx may be a factor in accelerated neuronal deterioration with aging. (AG04542).

510.1

MERKEL CELL POPULATIONS OF THE FOLLICLE-SINUS COMPLEX IN THE RAT HAVE A TEMPORAL DEVELOPMENTAL GRADIENT. T.E. Jones* and B.L. Munger* (SPON: R.D. Hartman). Dept. of Anat., M.S. Hershey Med. Ctr., Penn. St. Univ., Hershey, PA 17033.

Previous work in our laboratory has suggested that the two populations of Merkel cells associated with a follicle-sinus complex develop sequentially. To test this, rat pups of different developmental stages were obtained from timed pregnancies. Trigeminal axons innervate the superficial dermis in the mystacial region on GD13.0-GD13.5. By electron microscopy, Merkel cells were first seen associated with the vertical row sinus hairs G and H on GD16.5, and were present in the neck region of the vibrissae. On GD17.5, this population had separated from the neck region and were present in the outer root sheath. On GD17.5, nerves from the superficial nerve plexus approached the developing follicle from a lateral direction. On GD18.0, Merkel cells were identified in the epidermal rete ridge collar. The data suggest that differentiation of Merkel cells occurs subsequent to sensory innervation in the outer root sheath and the superficial epidermis. The presence of two distinct populations of a single cell type developing along a temporal gradient within a single structure may indicate a difference in behavior or function of the two cell populations. Supported in part by USPHS Grant #NS19462.

510.2

NEURON-RECEPTOR CELL INTERACTION DURING DEVELOPMENT OF THE INNER EAR: A HETEROCHRONIC GANGLION STUDY, T. R. Van De Water* and V. Galinovic-Schwartz*, (SPON: D. Spray) Depts. of Otolaryngology & Neuroscience, Laboratory of Developmental Otobiology, Albert Einstein College of Medicine, Bronx, N.Y., 10461.

It has been proposed that inner ear sensory receptors produce attractant fields that guide neurite outgrowth from the statoacoustic ganglion (SAG) to appropriate target sites within the developing labyrinth (Van De Water & Ruben, Acta. Otolaryngol. 95:470, 1983). This experiment tests the temporal limitations of the SAG in its ability to respond to these attractant fields. Statoacoustic ganglia were excised from 12, 13, 14 and 15 gestation day (GD) mouse embryos. This temporal series of SAG were implanted into explants of 12 GD inner ears, that had their SAG extirpated prior to implantation. All cultures were grown for six days in vitro, then fixed and processed for nerve fiber staining. Specimens were evaluated for the presence of neurites projecting from the implanted ganglia to inner ear sensory receptors that developed within the 12 GD otic explants. All of the implanted heterochronic SAG (i.e. 13, 14 or 15 GD SAG/12 GD OTOCYST) as well as the control homochronic ganglia extended neurities to sensory epithelium of both vestibular and auditory character. Neurites made contact with the bases of the hair cells in all of the sensory structures studied. These findings demonstrate that SAG neurons are capable of extending processes in response to otic attractant fields for an extended period during the embryonic development of this ganglion. They support the hypothesis that the onset and duration of receptor generated attractant fields may act as a controlling factor in establishing patterns of innervation within the developing inner ear.

(Supported by Grant NS08365 from the National Institutes of Health)

510.3

DO CENTRAL AFFERENTS INFLUENCE TARGET DIFFERENTIATION? T. Szabo, B.O. Bratton, D. Rouilly? C.N.R.S., Lab.Physiol. Nerveuse, Dept. Neurophysiol.Sensorielle, F 91190 Gif/Yvette, Zoologisches Inst., Univ. Regensburg, RFG.

The electromotor system was investigated in the weakly teleost Gnathonemus petersii in which a larval (LEO) and an adult (AED) electromotoneuron/electric organ complex develops successively during ontogenesis. Activity begins in the larval motor complex on day 9, 30 days before that of the adult motor complex which appears at d 40-45. Both larval and adult motor complexes are controlled by a common,spontaneously active bulbar command, the axons of which form the unique afferent pathway to the motoneurons. The different somatic position (trunk for LEO,caudal peduncle for AEO) allows easy deafferentation of the AEO while keeping intact the connection and activity of the LEO. Deafferentation was performed by spinal section anterior to the AEO Anlage at the 28th spinal segment, at different developmental stages of the larvae. Since differentiation of the AEO occurs between d20 and d40, deafferentation was carried out between d4 (hatching) and d20-23. Larvae had a survival time of 40 to 80 days. If spinal sections were performed between d15-23, the AEO showed a normally developed structure on day 45. However, sectioning at earlier periods resulted in incomplete development and a slowing down of the differentiation process. Reestablishment of functional central connections was never observed, up to 85 days, in spite of partial regeneration (or incomplete section) of the spinal cord.

510.4

DEVELOPMENT OF INTER- AND INTRA-HEMISPHERIC PROJECTIONS IN HAMSTERS. C. Hedin-Pereira*, R. Lent and S. Jhaveri*. Instituto de Biofísica Carlos Chagas Filho da UFRJ, Rio de Janeiro, RJ, 21941 and Massachusetts Institute of Technology, Cambridge, MA 02139.

The development of cortical afferentation by callosal and corticocortical fibers was studied using transport of WGA-HRP applied into the cortical wall of embryonic and postnatal hamsters. In frontal cortex, callosal axons emerge from the cortical anlage on E14 (conception = E1), cross the midline and arrive at the contralateral white matter by P1 (birth = P1). Commissuration proceeds from rostral to caudal: occipital axons reach the opposite hemisphere on P4. Axons wait in the white matter for a few days before innervating restricted target sectors of the gray matter. The zones of origin of the callosal projections are exuberant initially, but are subsequently trimmed to overlap with corresponding terminal fields.

Corticocortical projections follow the same developmental sequence as the callosal axons. Target innervation occurs much earlier for frontal than for occipital fibers. The distribution of cells giving rise to these connections is also initially exuberant and later restricted to the adult-like columnar pattern.

Thus inter- and intrahemispheric axons elongate, wait in the vicinity of the target, innervate terminal regions and undergo an adjustment in the distribution of cells wherein these projections originate.

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510.5

NEURONAL MORPHOGENESIS OF THE MEDIODORSAL NUCLEUS OF THE HUMAN THALAMUS:GOLGI STUDY. N.Zečević and J.Mojsilović* Dept.Neurophysiology, Inst.for Biological Res., Belgrade University, Belgrade 11000, Yugoslavia.

Golgi study of mediodorsal nucleus (MD) was performed in 10 human fetuses ranging in age from 12 gestational weeks (g.w.), crown-rump length (CRL=75mm) to 36 g.w.(CRL=480mm), one newborn and one adult. Brain tissue has been obtained through abortions performed for medical reasons or autopsies. In the group of youngest fetuses examined (12-14 g.w.) the area of the prospective MD was occupied by bipolar, branched bipolar and young multipolar neurons (mean values per neuron: total dendritic length, TDL, 272±17μm; soma area,s.a., 164±3μm; maximal diameter,dmax, 18.5±1μm). From 16 g.w. two types of MD neurons could be recognized: large (dmax>20μm) and small ones (dmax<20μm) presumingly projection neurons and interneurons, respectively. From this age to birth, TDL of both cell types combined, increased almost 5 times, s.a. increased two times and dmax 1.5 times. From birth to adult, TDL increased additional 2.2 times, while s.a. and dmax did not change significantly. In conclusion, from 16 g.w. to birth, MD neurons undergo major morphogenetic changes, but elaboration of dendritic tree continues into adulthood.

510.6

PROJECTIONS OF SUPERIOR CERVICAL GANGLION (SCG) NEURONS TO THE SUBMANDIBULAR GLAND AND IRIS IN RATS. J.I. Luebke and L.L. Wright, Dept. of Anatomy, BUSM, Boston, MA 02118

To determine whether single SCG neurons project to single or multiple target sites during different stages of development, the projections of SCG neurons to the submandibular gland (SMG) and iris were examined in 1, 8, and 24 week old rats using the retrograde tracers horseradish peroxidase (HRP) and Fluorogold (FG). Under anesthesia, five animals of each age were injected with HRP (2-3 ul) in one SMG and FG (1%, 2-3 ul) in the ipsilateral eye (anterior chamber). Also, several adult animals were injected with HRP in one SMG and FG in the other SMG, or HRP in one eye and FG in the other eye. After a survival of 3-6 days the rats were perfused and their SCGs sectioned and processed for the HRP reaction. The SCGs of rats injected in the ipsilateral SMG and eye contained no double labelled cells at any of the three ages. In SCGs of adults injected bilaterally in the eyes no double labelled cells were observed, however in adults injected bilaterally in the SMGs numerous double labelled cells were present. Only occasional neurons were labelled following application of either tracer to the SMG to simulate leakage. These data indicate that in the rat few or no SCG neurons project both to the iris and the SMG or bilaterally to the eye while some SCG neurons do project bilaterally to both SMGs. Supported by NIH grant NS2L577.

510.7

AXONAL TARGET TERRITORY INFLUENCES THE DEVELOPMENT OF DENDRITIC GEOMETRY IN THE ISTHMO-OPTIC NUCLEUS (ION) OF CHICK EMBRYOS - A GOLGI STUDY. S. Catsicas*, P. Blaser*, and P.G.H. Clarke* (SPON: J.P. Hornung). Institute of Anatomy, University of Lausanne, CH-1005, Switzerland.

In the mature ION, the neuronal perikarya are arranged in a convoluted lamina from which the dendrites project perpendicularly into the neuropil. By embryonic day 10 (E10), virtually all the dendritic trees are already highly polarized, but, unlike in the mature ION, most are directed ventralwards or ventromedialwards. Between E10 and E14, the dendrites change their direction of polarization so as to produce the adult pattern.

The afferents to the ION play little if any role in these events, because by the time they arrive in the ION (about E12) the first stage of repolarization is virtually complete. Moreover, tectal lesions at E10-E12 do not cause noticeable effects until after E14, when they are known to cause greatly enhanced neuronal death.

In contrast, the axonal targets seem to play an important role. Early removal of both eye primordia is known to cause the death of virtually all the ION neurons, beginning at E12-E13. But our present data, which are still being quantified, indicate that well before this, by E11, the dendritic trees in the target-deprived IONs are not so highly polarized as in unoperated embryos. This suggests that the axonal target may send an early retrograde signal to the ION neurons that modulates their dendritic geometry before they become dependent on the target for survival.

510.8

DEVELOPMENT OF SENSORY TRIGEMINAL AFFERENT PROJECTIONS IN *Rana Pipiens*. B.M. Rosenthal and K.E. Alley. Dept. Oral Biology, Ohio State Univ. Col. Dent., Columbus, OH 43210.

To determine if trigeminal sensory afferents undergo any reorganization during metamorphosis, ophthalmic (V1), maxillary (V2), and mandibular (V3) nerves were separately labelled with HRP and their central terminal arbors were examined in the hind brain and spinal cord of larval and adult frogs. In adults, afferents were arranged in the main sensory nucleus of V with V3 terminals medial and V1 and V2 terminals lateral. Terminals from V3 were also found in an area dorsal to the Vth motor nucleus corresponding to supratrigeminalis. The caudal extent of the spinal tract (Vsp) in adults was similar for each peripheral division (V1,2,3). A sparse projection was present at lumbar spinal root 8, but this was overshadowed by the much more dense projections to brachial and thoracic cord levels. Terminals primarily from the mandibular root ended in dense plexuses or tufts in the caudal medulla at the level of the hypoglossal nucleus. These tufts were also present in the contralateral medulla in adults. The basic pattern of sensory afferents was present in stage III larvae including the main sensory terminals and the brachial spinal projections, however, stage III tadpoles contained no Vsp axons caudal to the level of thoracic root 5, no terminals in supratrigeminalis, and no projections to the contralateral medulla. These observations suggest that larval development of the trigeminal system includes the expansion of previously existing axons into new terminal fields.

Supported by NIH grants DE 07620 (KEA) and DE 05516 (BMR).

510.9

ABNORMAL CUTANEOUS DIFFERENTIATION FOLLOWING LESIONS OF THE TRIGEMINAL GANGLION IN MONODELPHIS PUPS. B.L. Munger* and T. E. Jones* (SPON: K.E. Krebs). Dept. of Anat., M.S. Hershey Med. Cent., Penn. St. Univ., Hershey, PA 17033.

The present study was prompted by the observation that cutaneous innervation precedes differentiation of cutaneous appendages in both glabrous and hairy skin. We postulated that lesions of cutaneous nerves might perturb cutaneous differentiation and subsequently demonstrated this to be true following lesions of the caudal spinal cord in opossum pups. We have cauterized portions of the trigeminal ganglion in 1 day old Monodelphis domesticus pups and have studied the skin distal to the lesion in silver-stained serial paraffin sections. The epidermis and dermis close to the trigeminal lesion are hyperinnervated. The epidermis is hyperplastic and lacks hairs confirming findings following spinal cord lesions. The most striking changes were found in the eyelashes of both upper and lower lids. Eyelashes normally have a fixed angle with respect to the surface of the lid and are regularly and geometrically arranged. Following lesions involving the ophthalmic division of the trigeminal, the eyelashes of both upper and lower lids were irregularly spaced and had an incorrect angle with respect to the surface of the eyelid. These findings implicate afferent nerves as an important factor in the control of cutaneous differentiation. Supported in part by USPHS Grant #NS 19462.

510.10

THE USE OF SKIN EQUIVALENTS TO STUDY THE INFLUENCE OF SENSORY NERVES ON THE DIFFERENTIATION OF THE EPIDERMIS IN CULTURE. K.B. English and N. Stayner*. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

When collagen is mixed with dissociated fibroblasts, it is contracted into a dermis-like disk in 1 or 2 days. Keratinocytes can then be seeded on top of this tissue and grown to confluency, forming a skin equivalent (Bell et al., 1981, Science 211: 1052-1054). We have incorporated dorsal root ganglia from 21 day gestation fetal rats into dermal disks at the time of fabrication. The medium used was DMEM with glucose (600 mg/100 ml), supplements to promote keratinocyte survival [choleragen (10^{-10} M), hydrocortisone (0.4 ug/ml), and epidermal growth factor (10 ng/ml)], plus 10% fetal bovine serum and 10% heat inactivated horse serum. A 40:60 mixture of this medium plus KGM (Clonetics) was also employed. After the dermis was conditioned by the nerves it was seeded with keratinocytes. Three and 6 days later the tissue was fixed for histology and compared to cultures with non-conditioned dermis.

This system may prove useful to test the influence of various cell types on epidermal differentiation. (Supported by PHS grant 5 R23 NS23165-03)

510.11

PEANUT LECTIN STAINING IN THE MOUSE WHISKER-BARREL PATHWAY AND ITS MODIFICATION BY PERIPHERAL LESIONS AT DIFFERENT POSTNATAL AGES. J. Christensen* and T.A. Woolsey (SPON: R.S. Sohn) Division of Experimental Neurology & Neurosurgery & McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

Steindler, Cooper and their colleagues (J. Comp. Neurol. 249:157-169, 1986) showed that appropriately tagged plant lectins (associated with glia and components of the extracellular matrix) outline the whisker barrels of the cerebral cortex and whisker representations in the brainstem trigeminal nuclei and VB in the thalamus for a limited period in early postnatal life. The patterns change after lesions to the whiskers. We used HRP conjugated peanut agglutinin to label whisker representations in mice of known postnatal ages. In our hands, the appearance of the patterns is identical in sequence and time to that previously reported for a variety of stains including Nissl, CO, and several different specific axonal makers. The arrangement after lesions to the trigeminal nerve and its branches is as described with other markers except that the stain is denser where the lesions disrupt the whisker pattern. The obvious lack of staining in normal whisker representations, the sequence and its timing is consistent with the direction of pattern formation by projecting axons which appear to displace the lectin stained elements.

510.12

THE INFLUENCE OF MUSCLE CONTRACTILE ACTIVITY ON CROSS-STRIATIONS AND NUCLEI OF INNERVATED CULTURED HUMAN MUSCLE FIBERS (InnCHMFs). Y.C. Park,* V. Askanas, and W.K. Engel (SPON: C.K. HAUN). USC Neuromuscular Center. Los Angeles, CA 90017.

Adult human muscle fibers cultured aneurally in monolayer are poorly cross-striated, do not contract, and have nuclei located internally. We demonstrated that InnCHMFs are well cross-striated, contract continuously, and most of their nuclei are peripheral. To determine if either innervation or muscle contractile activity alone, or both together, is responsible for the development of cross-striation and the peripheralization of nuclei, we quantitated by light-microscopy of 3 um epon sections 1) area covered by cross-striation, and 2) number of peripheral vs. central nuclei, in InnCHMFs contracting for 4 weeks (Contr-InnCHMFs) vs. InnCHMFs paralyzed by tetrodotoxin for 4 weeks (Par-InnCHMFs) from the first day of their contractile activity. As compared to Par-InnCHMFs, Contr-InnCHMFs had: 1) 1400% ($p<0.001$) more of their total area covered by cross-striations; 2) 510% ($p<0.001$) more nuclei at the periphery; 3) 70% ($p<0.001$) fewer nuclei totally. In this culture system, innervation provides an essential signal to induce muscle contraction. However, development of cross-striation and peripheral migration of nuclei, a phenotype of adult muscle fibers, appears to be secondary to muscle contractile activity.

510.13

EFFECTS OF AN EXPERIMENTAL HINDLIMB GROWTH DISTURBANCE ON THE SCIATIC NERVE. C. Hildebrand, M. Westerberg and G.Y. Mustafa. Dept of Cell Biology, Faculty of Health Sciences, S-581 85 Linköping, Sweden.

The purpose of the study is to find out how a deficient length growth of the rat hindlimb influences the number of axons and the nodal spacing in the rat sciatic nerve. In neonatal rat pups the femoral and tibial epiphyseal cartilages on the left side were coagulated with the tip of a microcautery device. Postoperatively femoral and tibial length growth was markedly restricted on the left side, but the foot and pelvic region exhibited a normal longitudinal growth. Following a survival time of 6 months, the sciatic nerves were removed from both sides. Electron microscopic analysis of cross-sections from the nerve revealed that the content of axons was about 20% lower and that the myelinated fibres tended to be smaller on the growth retarded side. Light microscopic examination of teased preparations from pelvic and femoral levels of the left sciatic nerve showed that the relationship between internodal length and fibre diameter was normal in the pelvic segment and that the internodes were abnormally short in the femoral segment. These results suggest that the number of axons in the rat sciatic nerve adapts to a maldevelopment of the target that sets in at birth, and that internodal elongation during development proceeds according to the local growth in length of the nerve rather than to the length growth of the whole nerve.

510.14

COLLAGEN IN THE DEVELOPING AND INJURED ADULT MAMMALIAN CNS. R. Marchand, S. Woerly and A. Nadeau* (Spon: L.J. Poirier). Lab. Neurobio, Hop. Enfant-Jésus, 1401, 18e Rue, Québec G1J 1Z4.

Synthesis of collagen in the CNS has been reported in two circumstances (1) by the embryonic spinal cord epithelium (Cohen and Hay, 1971; Trelstad et al., 1973) (2) by primary human brain tumors (Rutka, 1987). We have investigated on the presence of collagen in the CNS by using the picrosirius red-polarization method (Junqueria et al. 1979). We visualized the presence of type I collagen in the developing rat brain on days 14.5, 15 and 16 of gestation, as a strongly birefringent red lining at the level of the internal limiting membrane of the ventricles. This collagenous membrane was occasionally associated to a non birefringent fibrinogranular substance that occupies the lumen of the ventricles and which stains with alcian blue suggesting a glycosaminoglycan-rich ependymal glycocalyx. Discrete radial type I collagen fibers were also seen in the mantle layer and at the level of the glial septum of the rhombencephalon. In the injured brain and spinal cord, collagen of type I was detected (1) in the lesion site as dense aggregations of thick bundles corresponding to the deposition of scar (2) at the lesioned interface under the form of types I-III orthogonally oriented copolymers. In addition, single, thin and green fibers of type III also occupied the neighboring healthy neural tissue. Our results show that the CNS tissue and/or the associated mesenchyme synthesize distinct genetic types of collagen in two circumstances: during organogenesis and following injury. The fact that types I and III collagens are often associated to morphogenetic and repair processes of organs suggest similar roles in the CNS: the setting of pathways for cell migration, the setting of a temporary framework for cell-cell and cell-matrix interactions, as well as the setting of a terrain for the initiation of regeneration and a role in the regulation of scar formation by the natural-occurring structural interactions between types I and III collagen fibers. (Supported by MRC and FRSQ).

510.15

NEONATE NUCLEUS BASALIS LESIONS RESULT IN CORTICAL CHOLINERGIC HYPOFUNCTION, AND COGNITIVE DEFICITS DURING ADULTHOOD. P.T. Jantzen[1]*, E.M. Meyer[2], W.J. Millard[3], A.J. Dunn[4], and G.W. Arendash[1]. (SPON: S.L. Swihart) Dept. of Biology[1], Univ. of South Florida, Tampa, FL 33620 and Depts. of Pharmacology[2], Pharmacodynamics[3], and Neuroscience[4], Univ. of Florida, Gainesville, FL 32610.

To eliminate developing cholinergic neurons within the nucleus basalis magnocellularis (nBM) region before they become functional (through their termination points in the neocortex), ibotenic acid was infused bilaterally into the nBM of 2 day-old male rats. Behavioral effects were investigated during adulthood and cortical neurochemical determinations were made at sacrifice, one year after lesioning. Histological analysis of the lesions indicated a restricted gliosis, essentially limited to the globus pallidus (including the nBM); neither the neostriatum nor the thalamus appeared to be damaged. Neurochemically, choline acetyltransferase (CAT) activity was found to be reduced by 25% in the frontal cortex of lesioned animals compared to sham-lesioned controls; cortical biogenic amine markers and neuropeptide (somatostatin and NPY) concentrations were unaffected. Behaviorally, lesioned animals showed a marked deficit in passive avoidance retention, as well as in the acquisition and retention of 2-way active avoidance behavior. These results indicate that a permanent cortical cholinergic hypofunction results from neonate nBM lesions, which chould be related to the cognitive impairments also observed. (Supported by a grant from the ADRDA)

510.16

THE INFLUENCE OF ULTRAHIGH MAGNETIC FIELDS ON CEREBRAL CORTICAL MORPHOLOGICAL DEVELOPMENT: A PRELIMINARY STUDY. M.C. Diamond, T.S. Tenforde*[1], E.R.Greer*, K.Hedges*, B.Steinke*, E. Davies*, J. Yu*, and D. Nguyen*. Dept. Physio-Anat. & Lawrence Berkeley Lab.[1], Univ. Calif. Berkeley, CA 94720.

Six 10-day-old male, unanesthetized, Long-Evans rats were housed in well-ventilated lucite holders and exposed for 17 min. to a 2.3 Tesla field (23,000 Gauss). Six 9-day-old males were similarly housed and exposed for 30 min. to a 7.5 Tesla field in a superconducting magnet. Six matched controls for each of the two experiments were placed in lucite containers in separate rooms. At 41 days of age, rats were anesthetized, perfused and brains removed. 20 micra, transverse, frozen sections were taken from the frontal, somatosensory and occipital cortex. On thionine stained sections, cortical thickness measurements of 9 areas/brain were made on microslide projected images (22.5X). All cortical measurements were done "blind", the codes broken only after all measurements were completed.

No significant differences in thickness were noted between the controls and experimental rats exposed to the 2.3 Tesla magnetic field even though 6 out of 9 areas measured were thicker. However, 8 out of 9 areas were thicker in the rats exposed to the 7.5 Tesla magnetic field with 3 areas statistically significantly different: area 4, right hem. (8%;$p<0.005$); area 10, right hem. (4%; $p<0.05$); area 3, left hem. (4%;$p<0.05$). The results suggest that exposure of neonatal rats to high-intensity magnetic fields promotes cortex development.

510.17

REGIONAL EFFECTS OF OPIOID RECEPTOR BLOCKADE ON CORTICAL THICKNESS IN ADOLESCENT RATS. J. Reyes*, D. Lewis*, and M. C. Diamond(SPON: N. Peterson). Department of Physiology-Anatomy, Univ. of California, Berkeley, CA. 94720

Investigators have demonstrated that specific regions of the cerebral cortex increase in thickness and cellular content when opioid receptors are blocked by Naltrexone. However, these studies examined preweaned rats, thus the role of opioids on CNS development are not known beyond this time. Our study examines the relationship between opioid receptor blockade, cortical thickness, and laterality in 9 regions of cerebral cortex in 41-day-old rats. Male and female Long-Evans rats were injected subcutaneously with either saline, 1, 10, or 50mg/kg of Naltrexone, an opioid receptor antagonist, for the first 41 days of postnatal life. On day 41, rats were sacrificed and morphometric analysis of the cortical regions were made on transverse, frozen, 40mu sections stained with a modified Thionine stain. Results indicated significant dose effects in the frontal and occipital cortical regions. The 10 and 50mg groups showed increases in cortical thickness. The 1mg group decreased or showed no change in comparison to saline injected controls. Cortical thickness in female rats increased significantly with each dosage of Naltrexone. The thickness in male rats increased in occipital cortex only at the 10mg dosage. At 50mg the occipital cortex decreased to below control levels. Changes in laterality patterns were noted.

510.18

EFFECTS OF OPIOID RECEPTOR BLOCKADE ON HIPPOCAMPUS DEVELOPMENT IN PREWEANED RATS. D. Fish*, J. Reyes*, T. Jensen*, D. Lewis* and M.C. Diamond (SPON: S. Roberts). Department of Physiology-Anatomy, Univ. of California, Berkeley, CA. 94720

Studies have indicated that regions of the CNS are increased in size and cellular content when opioid receptors are blocked by Naltrexone. Our study examines the relationship between opioid receptor blockade, size(thickness) and laterality in the hippocampus of 21-day-old rats. Male and female Long-Evans rats were injected subcutaneously with either saline, 1, 10, or 50mg/kg of Naltrexone, an opioid receptor antagonist, for the first 21 days of postnatal life. On day 21 rats were sacrificed and morphometric analysis of the hippocampus were made on transverse, frozen 40mu sections stained with a modified Thionine stain. Results indicated significant decreases in the hippocampal size at each dosage of Naltrexone. Male rats in each dosage group showed significant ($p<.01$) when compared to control(saline) rats. However, there was no significant differences between the dosage groups. There were no significant differences in laterality at any dosage. Female rats in the 1 and 10mg/kg group showed significant ($p<.01$) decreases in hippocampal size. Furthermore, females indicated laterality differences; as the dosage of Naltrexone increased the right hippocampus progressed from less than controls at the 1mg dosage ($p<.05$) to greater than controls at the 50mg dosage ($p<.01$). The left hippocampus remained less than controls for each dosage.

510.19

IMMUNOCOMPETENT CELLS IN HUMAN FETAL BRAIN CULTURES. F.Gremo,M.G.Ennas*,S.Torelli*,V.Sogos*, C.Marcello*&U.Lecca* Dept.of Cytomorph. & Inst. of Clin.Obst.Gynecol.,Sch.ofMed.,Cagliari,Italy

The hypothesis that glial cells can act as immunocompetent cells in the Central Nervous System has been recently advanced on the basis of in vivo and in vitro studies. However,data on humans are lacking. We had established human fetal brain cultures in our laboratory. Tissues were freshly dissected out of either spontaneous or medically induced abortions,cells were dissociated and seeded on polylysine pretreated dishes. Cultures were enriched in glial and neuronal cells. Contamination from other cells was minimal. Cells were fixed at different intervals and incubated with monoclonal antibodies against typical markers of B and T lymphocytes and of macrophages. Results showed that cells bore B_2,B_7,T_4,NH-NK,M_5,C_3B,Thy1 like antigens. Positivity for T_8,T_9,T_{11} was lacking. Double staining demonstrated that the most of these antigens were present on GFA-P positive astrocytes. Studies are in progress to test the physiological significance of these data.

TRANSPLANTATION: EYE

511.1

INNERVATION OF THE CORNEA BY ANTERIOR CHAMBER TRANSPLANTS OF NEONATAL TRIGEMINAL GANGLIA. C.S. Ogilvy*, K.R. Silverberg* and L.F. Borges. Neurosurgical Service, Massachusetts General Hospital, Boston, MA 02114.

The anterior chamber of the eye is a site which readily accepts neuronal tissue transplants and supplies the transplanted tissue with vasculature from the iris. The present study was designed to determine whether neonatal trigeminal ganglion transplants in the anterior chamber of adult rats would innervate the cornea. Transplants survived for at least 6 months after transplant. However, when these transplants were examined with retrograde fluorescent tracing studies and direct examination of corneal innervation using a gold chloride technique, no graft to cornea nerve fibers could be identified. Nor did the grafts innervate the cornea following host trigeminal nerve section.

Grafted tissue did innervate the host cornea when the neonatal trigeminal nerve fibers were inserted into the corneal stroma at the time of transplant. The extent to which host trigeminal innervation influences this sprouting is under study currently.

(Supported by a Dana Fellowship for Neuroscience and NIH Grant #: K08-NS00990)

511.2

INHERITED RETINAL DYSTROPHY: RESCUE OF PHOTORECEPTOR CELLS BY PIGMENT EPITHELIAL CELL GRAFTS IN THE RCS RAT. L. Li* and J.E. Turner. Dept. of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

The principal aim of research in animals that exhibit retinal degenerations, like the Royal College of Surgeons (RCS) rat, is directed at preventing, arresting or retarding photoreceptor cell (PRC) degeneration. We report that healthy retinal pigment epithelial cells injected into the subretinal space of RCS rats were found to rescue host PRC's from degeneration. RPE cells from 6 to 8 day old pigmented congenic RCS rats were injected into the subretinal space of the 26 day old RCS dystrophic rats. At P60 a time when most of the PRC's in RCS dystrophic rats have degenerated, its was found that the outer nuclear layer (ONL) of RCS dystrophic controls had been reduced to 1 to 2 cells in thickness. The same region of P26 dystrophic rats was 10 to 11 cells thick. In contrast, at P60 the presence of the RPE cell graft appeared to maintain the ONL tickness as in the P26 nongrafted RCS dystrophic control. The beneficial effects of the graft remain stable from at least neonatal life to young adulthood. This work represents the development of a successful therapeutic approach for the treatment of an inherited retinal dystrophy. It is also the first time that a cell transplantation procedure has been used to rescue a neuronal population that would normally die because of an inherited disease process. This research was supported by grants from NIH (EY04337), the National Retinitis Pigmentosa Foundation Fighting Blindness and Retinitis Pigmentosa International awarded to J.E. Turner.

511.3

TRANSPLANTATION OF ENRICHED CELL POPULATIONS DERIVED FROM IMMATURE RAT RETINA. M. del Cerro and H. H. Yeh. Dept. Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY. 14642.

We and others have successfully transplanted whole immature neural retina. Repair of damaged adult retina, however, may potentially be better approached by selective replacement of neuronal populations. To examine the feasibility of this more refined approach, donor retinal cells derived from selected layers of the immature retina were tested for their ability to survive and differentiate as intraocular transplants. Retinas from PN 4 Fisher 344 rat pups were isolated, treated briefly with trypsin, interposed as flatmounts between two sterile filter membranes and then cleaved at the level of the developing inner plexiform layer. Each of the two resultant adherent portions, an "outer" portion made up primarily of the neuroblastic mass and an "inner" portion which included the ganglion cells, was detached, suspended in 2 µl of medium and transplanted into the retinas of normal adult Fisher 344 hosts, or into those affected with phototoxic retinopathy. Sixteen days after transplantation the portion containing the neuroblastic cell mass had formed transplants consisting of photoreceptor cells, a plexiform layer, and a cell layer formed by inner nuclear cell profiles. These observations indicate that selected retinal populations are able to survive and to undergo histogenetic differentiation following transplantation. These enriched cell populations may be a useful source of donor tissue for attempts at repairing damaged retina.

Supported by EY05262 and Rochester Eye Bank.

511.4

TRANSPLANTATION OF IMMATURE DISSOCIATED NEURO-RETINAL CELLS ISOLATED BY FLOW CYTOMETRY. E. M. Pearlman, J. Leary*, M. del Cerro, M. F. Notter, C. del Cerro, and J. A. Olschowka. Departments of Neurobiology & Anatomy, and Pathology(*), University of Rochester Medical Center, Rochester, N.Y. 14642

Flow cytometry and cell sorting were used to characterize and isolate sub-populations of immature neural retinal cells for transplantation into adult retinas. Retinas from E22 rats were dissociated into a single-cell suspension. The cells were labeled with 6-carboxyfluorescein diacetate (cFDA), a measure of non-specific esterase, activity for sorting by an EPICS V multiparameter flow cytometer/cell sorter. Propidium iodide (PI), a DNA/RNA specific dye excluded by intact cells, was used to label damaged cells. Green cFDA fluorescence was measured between 515 and 545 nm, and red PI fluorescence was measured above 630 nm. Approximately 70-85% of cells were found to be viable based on PI dye exclusion. Cell size was measured using low-angle light scatter time-of-flight, a measure of cell diameter. These cell size measurements were in excellent agreement with cell size as measured by an IBAS image analysis system. Three sub-populations of neural retinal cells were seen based on cell size (or low angle light scatter) and cFDA staining. The brightest cFDA-positive cells were the smallest cells. The largest sized cells exhibited intermediate cFDA brightness. The medium sized cells had the dullest cFDA positivity. This group, which was the most numerous, was sorted into centrifuge tubes, labeled with Fast Blue, and used for transplantation into adult retinas. Fast Blue is a fluorescent marker shown by our laboratory (del Cerro et al., Neuroscience Abstracts, 1987)to label transplanted retinal cells for extended periods of time. Our results demonstrate the usefulness of the cell sorter for identifying and isolating developing retinal cell subpopulations suitable for intraocular transplantation. Supported by NEI grant 05262 and the Rochester Eye and Human Parts Bank.

511.5

INTRAOCULAR TRANSPLANTATION OF DEVELOPING RETINA INTO ADULT RETINAL DEGENERATION (*rd*) MICE. L.Q. Jiang, M. del Cerro, and C.M. Kalsow*. Dept. of Neurobiology and Anatomy and Ophthalmology, Univ.of Rochester, Med. Ctr., Roch., NY 14642.

The present era of intraocular retinal transplantation studies started in 1984 (del Cerro et al., ARVO 1984). Since that time our work and that of others has demonstrated that developing retinas from embryonic and perinatal donors can be successfully transplanted into host eyes with either normal or damaged retinas, and that transplantation can be effected even between animals of histoincompatable strains. All of these previous studies were performed using rats as experimental subjects. Presently we have successfully transplanted immature mouse retina into adult *rd* mutant mice. Newborn (PN 0) AKR/J mice served as donors and CBA/J adult mice as hosts which lost photoreceptor cells after birth as a result of *rd* mutation. Fragments of developing neural retina were transplanted into host retinas. The maximun survival period of animals allowed in these series was 15 days. Transplants grew into the host retina and subretinal space. Two interesting results were observed. First, the cells of AKR/J immature retina differentiated into photoreceptor cells in the CBA/J *rd* host's eyes. Second, S-antigen, a specific marker for photoreceptor cells, is expressed during the development of transplanted cells. It was seen in transplants examined at post transplantation (PT) 15 days, but not in those examined only at 3 PT days. The introduction of a murine system for retinal transplantation opens new posibilities, particularly through the use of visually defective mutant strains. Supported by NEI grant 05262 and the Rochester Eye Bank.

511.6

INTERSPECIES RETINAL TRANSPLANTATION. C. del Cerro*, L.Q. Jiang, and M. del Cerro (SPON: P. Shrager). Dept. of Neurobiology & Anatomy, Univ. of Rochester, Med. Ctr, Rochester, NY 14642.

Successful transplantation of developing neural retina into the retina of adult hosts of the same species has already been achieved by us and others. However, to our knowledge, retinal transplantation across species has not been reported. We wanted to test the feasibility of interspecies transplantation since it may permit the use of alternate sources of donor tissues and offer new oportunities to analyze host-donor cell interactions. Adult Fisher 344 albino rats were used in this study. Donors were newborn (PND 0) C57BL/6J mice. Strips of neural retinas were transplanted by injection into the posterior pole of host eyes. The transplants survived and resulted in the differentiation of ONL cells (with inner and outersegments attached to them), INL cells and a IPL. The transplants were well accepted up to 17 days after transplantation, the latest time point observed in these series. The host reaction was limited to a few macrophages surrounding the transplants. This mild reaction is comparable to that observed in the intra-species retinal transplants at the same postransplantation times. The transplants integrated closely with the host retinas, but still mouse donor and rat host cells can be differentiated from each other by their morphological features under LM and EM. These observations show that interspecies retinal transplantation is feasible and does offer interesting possibilities for the study of host-transplant interactions.
Supported by NEI grant 05262.

511.7

RABBIT RETINA TRANSPLANTS TO ADULT RABBIT RETINA.
M. Seiler, R. Aramant, B. Ehinger and A.R. Adolph.
Eye Research Institute and Harvard Medical School, Boston 02114, and University of Lund 22185, Sweden.

We report here for the first time the successful transplantation of rabbit embryonic retina to adult rabbit retina, using a modification of the Turner & Blair (1986) method. Young (4-6 weeks old) male albino rabbits were used as hosts. Under anesthesia, the dorsal scleral surface of the eye was exposed. A small (~1mm) incision was cut through sclera, choroid and retina and closed by 8-0 sutures. Retina from albino rabbit embryos (15 days after conception) was dissected free from surrounding tissues in cold PBS/1 mM Glucose and then placed into a small drop of PBS. It was taken up into a glass needle (0.2 mm dia.) injected into the lession site through a minimal lesion adjacent to the site.

Large grafts (~2 mm dia.) were found after 4 weeks survival, which consisted usually of many rosettes, visible as curls under the dissection microscope. In the graft rosettes, all retinal cell layers (with the exception of an inner limiting membrane) were observed. However, ganglion cells were not identified in the graft "ganglion cell layer". Graft photoreceptor cells frequently developed outer segments in rosettes. Often, a fusion between host and graft inner plexiform layers had taken place at the edges of the host retina lesion site. At least one specific class of retinal neurons, horizontal cells in the graft outer plexiform layers, was identified by an antibody against 160 kD Neurofilament.

511.8

NEURON-SPECIFIC MARKERS IN RETINAL GRAFTS TO ADULT RAT RETINA.
B. Ehinger, R. Aramant, M. Seiler, A. Bergström, J.E. Turner and A.R. Adolph.
University of Lund 22185, Sweden; Eye Research Institute, and Harvard Medical School, Boston 02114; Bowman Gray School of Medicine, Winston-Salem, NC 27103.

Embryonic rat E15 retinas were transplanted to an adult retinal lesion site as described (Turner & Blair, 1986). Eyes with grafts were fixed with 4% Paraformaldehyde after survival times of up to 7 weeks. Staining of frozen sections gave positive results for the following antibodies: ChAT (antibody provided by P.M. Salvaterra, CA), TH (antibody provided by P.R. Vulliet, CO), Neurofilament (160 kD), HPC-1 (specific for amacrine cells), and GABA. Amacrine cell bodies which were positive for ChAT (a cholinergic marker) could be seen in some 1 week old grafts and in all grafts at later stages. In 5 and 7 week old grafts, ChAT+ fibers were found in the graft inner plexiform layer at the border to the inner nuclear layer. TH+ processes of dopaminergic amacrine cells were stained in the graft from 1 week after transplantation. Horizontal cells in the graft stained weakly for Neurofilament one week after transplantation. From 2 weeks after transplantation, the staining intensity of horizontal cells in the graft outer plexiform layer corresponded to that of the host. The antibody HPC-1, directed against amacrine cells, stained specifically the inner plexiform layer (IPL) of adult rat retina. Weak HPC-1 staining could be seen in the graft IPL 2-3 weeks after transplantation. 5-7 weeks after transplantation, the intensity of staining was the same as in the host retina. An antibody against GABA clearly stained some cells in the graft (presumably amacrine cells) from 2 weeks after transplantation.

511.9

COGRAFTS OF RETINA AND TECTUM OR CEREBELLUM TO ADULT RAT RETINA. R. Aramant, M. Seiler and A.R. Adolph
Eye Research Institute and Harvard Medical School, Boston, MA 02114

Embryonic or neonatal rat retina has been shown to survive and differentiate when grafted to an adult retina (see Turner & Blair '86, Aramant et al. '88). Could ganglion cells develop and survive in these grafts deprived of their target? In attempting to answer this question, embryonic (E13 or E19) tectal anlage was grafted to adult rat retina, with or without a retinal cograft. As a control, some eyes received a corresponding cerebellar graft. The survival time was 4 weeks.

All E13 brain grafts survived, whereas E19 brain grafts mostly appeared to be rejected by the host. There was no difference in the high survival rate of E13 and E19 retinal grafts. E13 brain grafts fused with the host retina, pushing away host retinal tissue by their vigorous growth. E13 tectal and cerebellar grafts contained several classes of neurons corresponding to their origin, but they mostly failed to develop cell layers, and were filled with nerve fiber bundles (staining darkly for the 160 KD neurofilament (NF)). No clear effect of tectal grafts on the survival of ganglion cells (GC) in their retinal cografts was seen, but they seemed to attract ingrowth of host GC axons since heavily stained NF+ fiberbundles in the host retina were oriented towards the tectal graft. Neurons derived from the tectal graft migrated into the host inner plexiform layer even at distances of 1-2mm. Connections between the retinal and the tectal graft could be seen. Cerebellar (CB) grafts fused with the host retina, but no ingrowth of host GC axons was seen, and migration of CB neurons into the host retina was limited to the immediate environment of the CB graft. No connection between the retinal and the CB graft was observed.

511.10

ANATOMICAL AND BEHAVIORAL CORRELATES OF XENOGRAFT-MEDIATED PUPILLARY REFLEX R. D. Lund and H. Klassen* Dept. Neurobiol., Anat. & Cell Sci., Sch. of Med., U. Pittsburgh, Pittsburgh, PA 15261.

We are interested in the ability of neural transplants to make functional connections with host brains. We examine here whether cross-species transplants are able to drive a motor function in host rats through a normal pathway and whether the characteristics of the motor response correlate with the density of innervation of the appropriate brain region by the transplant.

We transplanted retinae from mouse embryos to the midbrain of neonatal rats which were unilaterally enucleated at the time of transplantation. After at least 3 weeks the remaining optic nerve was cut and the transplant exposed. Illumination of the transplant caused pupilloconstriction of the host eye, a response abolished by removal of the transplant or damage to the pupilloconstriction center, the olivary pretectal nucleus (OPN). The degree of constriction correlated directly with the intensity of illumination. We then examined how the responses correlated with patterns of host connections made by the transplant using mouse-specific monoclonal antibodies. The transplants projected only to areas of the brainstem normally innervated by the eye, including the OPN. The overall density of innervation did not, however, correlate predictably with the parameters of the pupilloconstriction response. Some animals with a brisk pupillary response had sparse innervation of the OPN while animals with heavily innervated nuclei sometimes showed a relatively slow response.

Thus ectopic neural xenografts are capable of making specific connections which can drive an appropriate response to a natural stimulus. While important in showing the ability of transplants to make specific functional connections, this study also provides a simple assay system for examining conditions which may improve the efficacy of host innervation by the transplant.

This research was supported by the Emmerling Fund of the Pittsburgh Fdn. and by NIH grant EYO5283 (RDL). HK is a Mellon Fellow.

511.11

RETINAL TRANSPLANTS CAN MAKE FUNCTIONAL CONNECTIONS WITH THE MATURE MAMMALIAN BRAIN. H. Klassen* and R.D. Lund (SPON: P. Munro) Dept. Neurobiol., Anat. and Cell Sci., Sch. of Med., Univ. of Pittsburgh, Pittsburgh, PA 15261

We have shown previously that fetal retinae transplanted into neonatal rats are capable of making the connections necessary for driving a pupillary reflex in the host eye in response to light. At birth the rat brain is still developing and therefore presents a favorable environment for fiber outgrowth and synaptogenesis. It is also important to determine whether such transplants will establish functional connections within the less plastic mature brain.

Fetal retinae taken from E13 Sprague-Dawley rats were grafted into the pretectal region of allogenic hosts which ranged in age from 36 to 42 days old. The contralateral eye was removed to reduce afferent competition between graft and host. The remaining optic nerve was later cut to eliminate all input from the host visual system. In many of the animals illumination of the transplant elicited a pupilloconstriction response in the host eye. The magnitude of the response however varied considerably. In some animals the host response was as brisk as that seen after transplantation into neonatal rats and, similarly, the degree of constriction was dependent upon the level of illumination of the transplant. Other animals showed extremely small changes upon stimulation. Approximately half of the animals in this study showed no stimulus-associated pupillary activity whereas similar transplants into neonates are associated with a failure rate of only 10% or less. Transplants which did exhibit a brisk pupillary reflex were consistently found to be imbedded in the pretectal area.

Athough the microenvironment of the mature mammalian brain seems to be less conducive to the establishment of graft-host connections than that of the developing brain, these results show that retinal transplants are capable of making the specific functional connections necessary to elicit an appropriate motor response in the host.

This research was supported by the Emmerling Fund of the Pittsburgh Fdn. and by NIH grant EYO5283 (RDL). HK is a Mellon Fellow.

511.12

TRANSPLANT AND HOST RETINAL INPUTS INTERACT TO DRIVE PUPILLARY REFLEX. J.D. Radel, H. Klassen* and R.D. Lund, Dept. Neurobiol., Anat. and Cell Sci., Univ. of Pittsburgh Sch. Med., Pittsburgh, PA 15261.

Retinal transplants have been shown to be capable of driving a pupillary reflex in rats after elimination of host optic input. We investigate here whether transplanted retinae can also drive the pupillary reflex in the presence of an intact host visual system.

Embryonic (E13) rat retinae were transplanted into the midbrain of one day old rats. In one group of animals one eye was removed at the time of transplantation, while in a second group both eyes were left intact. Beginning three weeks postnatally, the skull overlying the transplant was removed and the transplant exposed. Illumination of the transplant produced host eye pupilloconstriction, although the degree of constriction varied across animals. The transplant-mediated pupillary reflex was observed in both eyes of the two-eyed hosts and was in all cases obtainable immediately upon exposure of the transplant. By using 2 separate light sources it was possible to vary independently the intensity of host and transplant illumination. Photic stimulation of either the transplant or the host elicited pupilloconstriction, the extent of which was directly correlated with the luminance intensity. When host eye stimulation was maintained at an intermediate level, illumination of the transplant resulted in further constriction of the host pupil.

These results indicate that intracranial retinal transplants, which receive minimal photic stimulation until they are sugically exposed for testing, can make usable connections even when forced to compete for synaptic sites with the active, intact host visual system. Furthermore, transplant encoded luminance information received by the host brain is not overwhelmed by activity within the normal visual pathway, but is instead combined with information from host retinae to produce an additive effect on pupilloconstriction.

This research was supported by NIH grants EY05962 (JDR) and EY05283 (RDL). HK is a Mellon Fellow.

511.13

CORTICAL ACTIVITY CAN BE ELICITED BY LIGHT STIMULATION OF RETINAL TRANSPLANTS. S. Craner*, J.D. Radel and R.D. Lund (SPON: P. Land) Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Previous work in this laboratory has demonstrated that retinal transplants are capable of evoking unit activity in the superior colliculus in response to photic stimulation. The present study examines the ability of retinal transplants to evoke activity in the visual cortex of host rats.

Retinae were removed from embryonic (E13) rats and transplanted into the midbrain of postnatal (P1) rat hosts. One of the host's eyes was removed at that time to enhance the transplant projection. Between 4 and 8 weeks postnatally, the skull overlying the brainstem was removed. The presence of a transplant-mediated pupillary reflex was used to assay for functional transplant-host connections. With the eye shielded, the brainstem was illuminated in an effort to drive the pupillary reflex in the remaining host eye via the transplant. The right and left visual cortices were then exposed in animals exhibiting transplant-mediated pupillary reflexes.

Evoked potentials and multiunit activity were recorded in visual area 18 of the cortex ipsilateral to the remaining host eye in response to photic stimulation of the transplanted retina. These responses were graded with respect to the intensity and duration of the stimulus. Contralateral cortical responses to illumination of the host eye were compared to those responses mediated by the transplant. To confirm that the evoked activity was truly transplant-mediated, the host eye was shielded or removed. Following the testing, the presence of a transplant was confirmed histologically.

These results demonstrate that photic stimulation of retinal transplants is capable of eliciting appropriate activity in the visual cortex, presumably via transplant-mediated activity in the superior colliculus and lateral posterior nucleus of the thalamus.

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511.14

FORMATION OF A NEURAL CIRCUIT BY TRANSPLANTED REGIONS OF RAT VISUAL SYSTEM. H.F. Zhou*, J.S Lund and R.D. Lund (SPON: S.K. Wolfson,Jr), Dept. Neurobiol., Anat. and Cell Sci., Univ. Pittsburgh, Pittsburgh, PA 15261

Previous studies show that retinae grafted to the rat cortex send projections to co-transplanted diencephalon. To determine if transplanted diencephalon also projects to the cortex and if a neural circuit could be established by several transplanted regions, diencephalon was dissected from E14 CD-1 mice and was placed in cortex of one-day old (P1) Sprague-Dawley albino rats. Host brains were fixed with 4% paraformaldehyde 3-9 weeks after transplantation. Monoclonal antibodies to mouse neuron surface antigens (M6 & M4) were used to demonstrate projections from the grafts. Transplants placed near visual cortex projected to layers I, IV and VI in the form of patches. In a further study, E14 mouse diencephalon and E16 rat occipital cortex (or the reverse: E14 mouse cortex and E16 rat diencephalon) were co-grafted into the right superior collicus (SC) of P1 rats. The left eyes of the rats were removed 36 hours prior to fixation at three or more weeks of age. The projection from host eyes to transplanted diencephalon was demonstrated by the Fink-Heimer method. Degenerating optic axon terminals were found in the host SC and in parts of the transplanted diencephalon. Using antibody staining, we found that transplanted diencephalon projected to co-transplanted occipital cortex, and cortex also projected back to the entire transplanted diencephalon.

The results indicate that co-transplanted brain regions can make appropriate connections with each other and with host neural structures. Supported by NIH grant EY05283.

511.15

RETINAL GRAFTS IMPLANTED INTO THE ADULT EYE LESION SITE STIMULATE THE DAMAGED HOST RETINA. Laedtke, Thomas W.* and James E. Turner (SPON: D.J. Goode). Dept. of Anatomy, Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, NC 27103

This study tests the potential for wound repair in the host retina by retinal implants. More specifically, it examines the effects of retinal grafts on the local degeneration which follows lesion surgery. Adult Sprague-Dawley rats received a lesion incision made through the sclera, choroid, and retina. In addition, a second group of animals also received implants of embryonic day 15 retinal tissue, placed into the host lesion site. Animals were sacrificed 28 days following surgery and tissues were analyzed by light microscopy to evaluate the thicknesses of the optic fiber layer-ganglion cell layer (OFL-GCL), inner plexiform layer (IPL), inner nuclear layer (INL), and outer nuclear layer (ONL), as well as the ganglion cell density (GCD). These measurements were taken in a 1000 μm region peripheral and central to the lesion. Controls not containing retinal implants exhibited dramatic reductions in individual layer thicknesses peripheral to the lesion site. Similarly, the GCD decreased by 50% in this region compared to unlesioned control animals. The presence of a retinal implant, however, resulted in a reduced degenerative response in layer thicknesses peripheral to the lesion and a dramatic increase in the thickness of the OFL-GCL, INL, and ONL central to the lesion site. This stimulatory effect was also evident in GCD as ganglion cell loss in the peripheral retina was reduced by 20% when compared to lesioned controls. Thus, retinal implants appears to elaborate putative diffusable factors to induce a 'rescue' effect on the degenerating host retina. Supported by NIH Grant No. EY04377.

511.16

TRANSPLANTATION OF RETINAL PHOTORECEPTORS TO DYSTROPHIC RETINA. S.E. Hughes* and M.S. Silverman* (SPON:D. Parkinson), Central Institute for the Deaf and Washington University School of Medicine, St. Louis, MO 63110.

Inherited retinal degeneration afflicts a variety of animals, including humans. Several established animal models exist for inherited retinal dystrophy and retinitis pigmentosa, including the RCS rat and the rd mouse. These strains provide model systems in which it is thought that the deficit resides in the photoreceptor (the rd mouse) or is related to the pigment epithelium (the RCS rat). We have devised methods for isolating the outer plexiform layer from the retina and transplanting the resulting photoreceptor layer to the subretinal space (Silverman and Hughes, Invest. Ophthalmol. Vis. Sci. Suppl. 28: 288; Soc. Neurosci. Abstr. 13: 1301). We transplanted neonatal (8 day old) photoreceptors from nondystrophic congenics to the subretinal space of adult dystrophic animals. For definitive identification of the transplanted cells as photoreceptors we used a monoclonal antibody specific to opsin (RET-P1 from C. Barnstable). With H&E staining the transplanted cells are easily distinguished from any residual host photoreceptors.

In the rd mouse, the transplanted photoreceptors have been found to survive for at least 4 weeks. This is significant because photoreceptors of the rd show degeneration after 2 weeks, with few remaining after 3 weeks. The survival of the transplanted photoreceptors supports findings that the deficit within the rd retina is endogenous to the rd photoreceptors themselves. To our surprise, we have also found that transplanted photoreceptors survive in the RCS rat. This may be due to their limited production of outer segments. It is the failure of the pigment epithelium to remove the accumulation of shed outer segments which is thought to lead to photoreceptor elimination in the RCS retina.

Supported by grants from the National Retinitis Pigmentosa Foundation and the Monsanto Company.

511.17

TRANSPLANTATION OF HUMAN PHOTORECEPTORS TO LIGHT DAMAGED RETINA. M.S. Silverman* and S.E. Hughes* (SPON: C. Blazynski). Central Institute for the Deaf and Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Previously we reported successful transplantation of the intact photoreceptor layer from the developing as well as mature rat retina to adult albino rats with light-induced loss of photoreceptors (Silverman and Hughes, Invest. Ophthalmol. Vis. Sci. Suppl. 28: 288; Soc. Neurosci. Abst. 13:1301). These results prompted us to ask whether mature human photoreceptors from adult eye donors might also be transplantable.

Photoreceptors were taken from the retina of donated human eyes (obtained from the MO Lions and St. Louis Eye Banks) following corneal removal. Hosts were adult albino rats (immune suppressed or immune competent) exposed to constant illumination for four weeks which decimates host photoreceptors while leaving the remaining retina intact. The isolated photoreceptor layer was transplanted using a transcorneal approach to the subretinal space. The retina reattaches to the back of the eye with the transplanted photoreceptors interposed between the retina and the underlying pigment epithelium. With immune suppression photoreceptor transplants were successful at all survival times so far examined showing apparent physical integration with the host retina and maintaining morphological features of the outer nuclear layer. The transplants stained positive for antiopsin antibody RET-P1 (C. Barnstable) identifying the transplanted cells as photoreceptors and further suggesting that they may be capable of light transduction. In contrast, transplants to immune competent hosts showed signs of rejection within one week of transplantation. Sham operates showed no repopulation of the host retina with photoreceptors.

These results show that human photoreceptors can be transplanted and, significantly, that mature photoreceptors can be transplanted while other neurons apparently must be developmentally immature for successful transplantation.

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511.18

FETAL EYE AND SCIATIC NERVE SEGMENTS AS BRIDGES BETWEEN HOST ANTERIOR EYE CHAMBER AND FOREBRAIN. B. H. Hallas and M. F. Zanakis. New York College of Osteopathic Medicine, Old Westbury, New York 11568 and American BioInterface Corporation, New York, New York 10276.

Various age rat fetal eyes (e. 14-21d.) were removed intact and sutured to the proximal segment of an adult rat sciatic nerve from the same strain. The entire fetal eye/sciatic nerve combination was then inserted into the anterior eye chamber of adult hosts. The distal portion of the sciatic nerve bridge was inserted into the host's forebrain. Simultaneously as the fetal eye/sciatic nerve bridge was implanted, the ipsilateral optic nerve was crushed and dry ice applied to the area of the crush as close to the host retina as possible.

Thirty days post-implantation a 40% solution of HRP was injected intraocularly. In a second group of identically implanted animals the sciatic nerve segment was transected midportion and HRP applied directly to the transected ends. Forty-eight to seventy-two hours after the application of HRP, all animals were sacrificed. In all host animals, regardless of the age of the fetal eye, the implant survived, grew, differentiated and the label patterns in the host forebrain and implant were quantified. This implant/peripheral nerve technique is a useful model for the study of CNS regeneration and repair.

NEURAL CONTROL OF IMMUNE SYSTEM III

512.1

SUPPRESSION OF PROLACTIN BY ORAL CYSTEAMINE TREATMENT SUPPRESSES IMMUNE RESPONSES IN MICE. E.W. Bernton*, J.W. Holaday and H.U. Bryant., Dept. Med. Neurosci., Walter Reed Army Inst. Res., Washington, D.C. 20307-5100.

Suppression of pituitary prolactin (PRL) secretion by bromocryptine suppresses various immunologic responses in mice. Since cysteamine HCl (CYS) suppresses pituitary PRL secretion by mechanisms unrelated to dopamine-agonist effects, we examined its effects on immune and neuroendocrine correlates. In a time course study, male C3H/HeN mice received CYS (200 mg/kg/day; po), and were then sacrificed for determination of serum PRL and corticosterone (CS), and mitogen-induced lymphocyte blastogenesis. Serum PRL levels were unaffected after 1 day of CYS, but were suppressed following 2, 3, and 4 days (46, 51 and 47% of control, respectively). Serum PRL remained suppressed 2 and 3 days after CYS treatment (73 and 52%, respectively). Lymphocyte blastogenesis in response to 1 µg/ml concanavalin A and 2.5 µg/ml lipopolysaccharide were suppressed with 3 or 4 days CYS. Reduced blastogenic responses were noted at 2 and 3 days after stopping CYS and had recovered by 4 days. CYS treatment did not increase CS levels, suggesting this treatment was not a nonspecific stressor. Coincident treatment with PRL (50 µg/day) attenuated immunosuppression in CYS-treated mice. Cysteamine treatment also suppressed splenocyte reactivity in a mixed lymphocyte reaction and antibody secretion in a plaque-forming assay. These studies suggest that suppression of PRL secretion mediates the immunosuppressive effects of CYS, further supporting the role of PRL as an important immunotrophic hormone.

512.2

ENHANCED BLASTOGENESIS OF HUMAN LYMPHOCYTES BY PROLACTIN. S. Kennedy, W.B. Malarkey*, D.M. Shaut* and R. Glaser*. Ohio State University Coll. of Med., Columbus, OH 43210.

Recent developments in neuroimmunology have pointed to the interplay between the nervous, endocrine and immune systems. For example, lymphocytes possess receptors for a number of neurohormones, including prolactin and growth hormone.

In the present study, human lymphocytes were cultured with either human or ovine prolactin in combination with the mitogens phytohemagglutinin (PHA) or concanavalin A (Con A). Optimal culture conditions for mitogen stimulation were determined in preliminary studies. Cultures were harvested at 96 or 120 hours; blastogenesis was measured by tritiated thymidine incorporation. In contrast to prolactin or mitogen alone, treatment of cells with PHA or Con A in combination with prolactin resulted in a dose-dependent enhancement of blastogenesis. Both human and ovine prolactin were effective in their additive effects with mitogen. We are currently examining the effects of prolactin inhibitors on alterations of blastogenesis by prolactin.

The data thus far indicate that T-lymphocyte replication in response to mitogens may be influenced by levels of prolactin. These data are in agreeement with an earlier report examining prolactin function in the rat (Hiestand, et al, Proc. Natl. Acad. Sci., 1986, 83, 2599-2603), and suggest that prolactin may be a critical neuroimmune modulator.

512.3

SOMATOSTATIN (SS) AND ITS mRNAS ARE DECREASED IN GVHD. L. Chen* and W.S.T. Griffin (SPON: T.M. Badger). Depts. Ped. and Anat., U. AR Med. Sci., Little Rock, AR 72205.

Immunohistochemistry, Western immunoblotting, and Northern hybridization were used to analyze relative levels of immunoreactive SS and SS mRNA in the brain of immunoincompetent neonatal rats, bearing allogeneic-lymphocyte grafts that cause graft versus host disease (GVHD). GVHD levels were compared to those from littermates receiving alloantiserum treatment (AAS) to cure their GVHD or from uninjected littermate controls (C). The numbers of SS-immunoreactive cells in temporal lobe cortex and hippocampus regions were less in GVHD than in AAS or C as were the relative levels of both the neuropeptide and its encoding mRNAs. Hippocampectomy or experimentally decreased CSF levels of SS results in an increase in the release of hypothalamic corticotrophin releasing factor (CRF), pituitary adrenocorticotrophic hormone (ACTH), and corticosterone (CORT). As CORT titers are regulated by SS and CORT is immunosuppressive, we hypothesize that the elevation of CORT titers in GVHD is related to the decrease in SS in GVHD brain and that the elevated CORT contributes to the profound immunodeficiency syndrome that is associated with GVHD. AAS reverses both the elevation of CORT and the decrease in the number of SS-immunoreactive cells, suggesting that GVHD-related agent(s) regulate SS levels in brain cells rather than cause the loss of SS-immunoreactive cells.

512.4

AGE AND ROUTE SPECIFIC DIFFERENCES IN RESPONSE TO IL-1 L. W. Martin*, L. B. Deeter* and J. M. Lipton (SPON: G. Moushegian). Dept. of Physiol., The Univ. Tex. Southwestern Med. Ctr. at Dallas, Dallas, TX 75235.

Many aspects of the acute phase response (APR) are muted in the elderly, and the reasons are unknown. Administration of the cytokine IL-1 induces many components of the APR, implicating IL-1 as a mediator of the APR.

To determine if the route of administration or age affect the acute phase response to IL-1 we administered IL-1 intracerebroventricularly (ICV), or intravenously (IV), to young and old female rabbits and monitored fever, circulating white blood cells (WBC) and neutrophils, and plasma levels of α-MSH, a neuropeptide that antagonizes actions of IL-1.

ICV IL-1 in young rabbits caused fever, but no significant changes in WBC, neutrophils or α-MSH. ICV IL-1 in old rabbits caused no fever and modest increases in neutrophils and α-MSH. IV IL-1 in young rabbits caused fever, increased WBC, neutrophils and α-MSH. IV IL-1 in old rabbits did not cause fever nor increases in WBC, neutrophils or α-MSH. The results indicate that: (1) the response to peripheral and central IL-1 is reduced in aged animals, and (2) that the effect of IL-1 on the APR in young animals is greater when it can act both centrally and peripherally after IV injection.

Supported by NIH GRANT AG00109 and NINCDS GRANT NS10046.

512.5

OCCURRENCE OF β-ENDORPHIN AND RELATED PEPTIDES IN STIMULATED AND UNSTIMULATED HUMAN PERIPHERAL BLOOD LEUKOCYTES. A.D. Van Woudenberg*, V.M. Wiegant*, C.J. Heijnen*+ and D. De Wied*, Rudolf Magnus Institute of Pharmacology, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands, + Dept. Pediatric Immunology, University Hospital for Children and Youth, Utrecht, The Netherlands.

Recently it has been shown that human peripheral blood leukocytes (PBL) can produce POMC-derived peptides, particularly when stimulated by virus or corticotropin releasing factor (CRF). The present study demonstrates that endorphin immuno-reactivity (IR) occurs also in unstimulated PBL. Peptides were extracted from PBL isolated from freshly obtained blood of healthy volunteers, by heating in 1M acetic acid and homogenization. The extracts were analyzed in three radio-immunoassay systems specific for: 1) β-endorphin (βE), 2) α-endorphin (αE) and 3) γ-endorphin (γE). With each of these assay systems IR was detectable in all the extracts analyzed. The IR amounted to 200, 60 and 10 pg/10^7 cells for β-, α- and γ-E respectively. When PBL were cultured in the presence of Con A for 48 hrs, the βE content of cell extracts had increased twofold compared to nonstimulated cells. PBL were separated in subsets enriched in T-cells, B-cells and monocytes. In all three subsets, βE IR was found. The concentration in T-cells however appeared to be lower than that in the non T-subsets. The present data show that human PBL contain endorphin IR and suggest that such cells may express POMC not only under stimulated, but also under normal conditions.

512.6

CHEMOTACTIC ACTIVITY OF OPIOID AND NON-OPIOID FRAGMENTS OF BETA-ENDORPHIN. P.Sacerdote, L.Palazzolo* and A.E.Panerai. Dept. of Pharmacology, School of Medicine, University of Milan, Italy.

ß-Endorphin (ßE) has been shown to modulate several immunological functions. Some of these effects are not mediated by a classical opioid receptor, as they are not reversed by naloxone. We analyzed the monocyte chemotactic activity of opioid (N-terminal) and non opioid (C-terminal) fragments of ßE. Both N-terminal and C-terminal fragments are chemotactic. ßE 1-31 is the more potent: peak activity at 10^{-12} M, CI=3: chemotaxis is reversed by naloxone. The N-terminal sequences ßE 1-17, 1-16, and 1-27 are as potent as the C terminal fragments ßE 6-31 and N-acetil, with a maximum CI of 2.5 at 10^{-11} M. Only the N-terminal fragment induced chemotaxis is reversed by naloxone, suggesting that the C-terminal fragments bind a receptor different from the classical opioid one. This analysis suggest that ßE can interact with human monocytes at two different sites, and suggest a physiological role for the circulating form N-acetil ßE function was still unknown.

512.7

ß-ENDORPHIN REVERSES PGE_1 SUPPRESSION OF RAT T-CELL MITOGEN-INDUCED PROLIFERATION. L.M. Hemmick and J.M. Bidlack. Dept. of Pharmacology, Univ. of Rochester Sch. of Med. and Dent., Rochester, NY 14642.

Prostaglandins of the E series (PGE) have been shown to increase cAMP levels, decrease phosphatidylinositol phosphate hydrolysis, and inhibit mitogen-induced proliferation of T-lymphocytes. In brain, ß-endorphin (BE) inhibits cAMP levels, while in rat thymocytes, BE enhances mitogen-stimulated Ca^{2+} uptake (Hemmick, L.M. and Bidlack, J.M., Life Sci. 41:1971 (1987). This study addressed the question: Can ß-endorphin and other opioid peptides reverse the PGE suppression of lectin-induced T-cell proliferation? Cervical and mesenteric rat lymph node cells were cultured for 72 hr with either phytohemagglutinin (PHA) or concanavalin A (Con A) in the presence of PGE_1 and opioid peptides prior to the measurement of proliferation by ^{3}H-thymidine incorporation into the DNA. BE 1-31 reversed PGE_1 suppression of PHA and Con A-stimulated proliferation. The reversal was titratable from 10^{-8} M to 10^{-5} M, with a maximal enhancement of 79 ± 13% at 10^{-5} M BE 1-31. [D-Ala^2]met-enkephalin (DAME) also reversed the PGE_1 suppression. Neither BE 1-31 nor DAME affected basal or mitogen-stimulated proliferation of T-cells. These data suggest that BE and DAME may regulate T-cell proliferation by counteracting the inhibitory actions of PGE and other compounds which stimulate cAMP levels. [This work was supported by grants DA 03742, DA 05302, and DA 07232 from the National Institute on Drug Abuse.]

512.8

OPIOID MODULATION OF INVERTEBRATE HEMOCYTE MIGRATION. P. Cadet*, L. Simmons*, J. Sinisterra*, G. B. Stefano, X.Zhao* and M.K. Leung. CTR for the Study of Aging, SUNY/Old Westbury, Old Westbury, N.Y. 11568, USA

The interaction among the immune, nervous and endocrine systems is due to the close chemical relationship between their messenger substances. In Mytilus edulis (Bivalvia), where opioid substances are known to exist, the branchial nerve was severed to determine if the immune response may involve opioid substances as noted in mammals. This area was observed via histofluorescence, immediately, 2 hours and 10 hours following the surgery. This treatment evokes a cellular immune response as noted by the directional migration of specific serotonin-containing hemocytes to the damaged nerve. Injection of DAMA (10 ul, 10^{-6} M) into an area close to the served nerve resulted in a migration of the hemocytes into this vicinity as opposed to the lesioned neuron. Naloxone injection into the immediate severed nerve area appeared to inhibit/block the specific hemocytes from adhering to the severed branchial nerve. This opioid chemotactic activity appears to be select since epinephrine, norepinephrine, dopamine, serotonin, substance P, insulin, octopamine and FMRFamide did not induce the cellular clustering. The results suggest that opioid interaction with "blood" cells arose early in the course of evolution. **Supported by grants NIH-MBRS 08180 & ADAMHA-MARC 17138.**

512.9

POTENTIATION OF IMMUNE RESPONSIVENESS FOLLOWING PITUITARY GLAND TRANSPLANTATION. R.J. Cross and T.L. Roszman (SPON: L.Boyarsky, Dept. of Microbiology & Immunology, Univ. of Kentucky, Lexington, KY 40536-0084.

Recent reports have shown that the pituitary hormone, prolactin is required for immune function. Administration of the dopamine agonist, bromocryptine, has been shown to impair macrophage function and humoral immune responsiveness, while transplantation of syngeneic pituitary grafts (SPG) in immunocompromised, hypophysectomized animals restores immunocompetence. These data extend these previous reports by showing that SPG augment the humoral antibody response of normal mice. SPG were transplanted under the kidney capsule of C57Bl/6 mice and 10-14 days later the mice were immunized with sheep red blood cells (SRBC). The resulting antibody response shows that mice with SPG have significantly enhanced IgM and IgG plaque-forming cell responses. Serum prolactin levels in these mice are also elevated. Similarly, mice with two SPG display antibody responses that are elevated compared to mice with one SPG. SPG however do not increase the number of nucleated cells in the spleen or alter the percentage of B-cells,T-cells or T-cell subsets (Lyt 2 or L3T4). Immunization of SPG-mice with the T-cell independent antigen, TNP-LPS, indicates that enhancement of immune responsiveness can occur independent of T-cell Function. (Supported by USPHS grants NS22512 and NS17423.

512.10

TEMPORAL DISSOCIATION BETWEEN STRESS-INDUCED CHANGES IN NEUROENDOCRINE AND IMMUNE SYSTEMS. C.M. Flores*, M. Hernandez*, K.M. Hargreaves* and B.M. Bayer* (SPON: S. POTOLICCHIO). Dept. of Pharmacology, Georgetown Univ. Med. Ctr., Washington, DC 20007.

These studies examined the temporal relationships between stress-induced changes in the neuroendocrine and immune systems. Rats were implanted with jugular catheters. Following 24 hrs, animals were either stressed by immobilization or placed in individual cages. Blood samples were drawn at 0, 30, 60, 90, 120, 180 and 240 minutes and mitogen-stimulated lymphocyte proliferation and corticosterone, β-endorphin and prolactin plasma levels were determined. Basal corticosterone levels (83 + 31.3 ng/ml) increased 4 to 5 fold in stressed animals within 30 min and remained similarly elevated for 240 min. Basal β-endorphin levels (138 + 23.3 fmol/ml) increased 4 fold by 60 min and declined to basal levels within 240 min. Basal prolactin levels (12.1 + 1.9 ng/ml) maximally increased within 30 minutes (141 + 7) and declined thereafter. No significant differences were found in blood lymphocyte responses of restrained and control animals. In vitro studies showed that only corticosterone was inhibitory when added directly to lymphocyte cultures. These results suggest a temporal dissociation between the increase in corticosterone and suppression of lymphocyte activity during the response to stress.

(Supported by NIH Grant No. DA 04358)

512.11

MODULATION OF ALLOGENEIC AND AUTOLOGOUS MIXED LYMPHOCYTE REACTIVITY BY NERVE GROWTH FACTOR. L.W. Thorpe and J.R. Perez-Polo. Dept. H.B.C.&G., Univ. Tex. Med. Br., Galveston, TX, 77559.

We and others have demonstrated that nerve growth factor (NGF) plays a modulatory role in immune function. The mixed lymphocyte response (MLR) is a measure of the ability of lymphocytes to recognize and destroy foreign or transformed cells. The autologous mixed lympho- cyte reaction (AMLR) to self-antigens is thought to be critical in the maintenance of immune tolerance. The effects of NGF on MLR and AMLR activity was examined using one-way cultures of rat (LEWvsBN) splenocytes (MLR) and autologous T vs nonT cell (LEW) cultures (AMLR). The cells were incubated (5-6 days) with or without NGF (0.1-10 ug/ml) and assayed for ^{3}H-thymidine uptake. In the AMLR increased proliferation was seen for all concentrations of NGF tested, however a significant increase in DNA synthesis was observed in the MLR only at the highest NGF dose. These results suggest a differential NGF effect on the activation of responding lymphocyte subsets and a response dependent on the type of antigenic stimulus. These observations streng- then our hypothesis that NGF plays a signal role in neuro-immune interactions. Supported by ONR-RR 04108.

512.12

EFFECTS OF HUMAN AND RODENT INTERFERON ON BEHAVIOR. L.S. Crnic and M.A. Segall* Departments of Pediatrics and Psychiatry, University of Colorado School of Medicine, Denver, CO 80262.

Interferon (IFN) is known to produce nausea in humans. Therefore, to determine whether human (IFN) was effective in rodents, rats were subjected to a conditioned taste aversion (CTA) procedure. Results showed no evidence of CTA to 150 nor 600 units/g of human recombinant IFN alpha. IFN did not block LiCl CTA. No effects of human IFN upon radial maze learning in rats were seen. We had shown that this same IFN accelerates the onset of REM sleep in primates (Reite et al, Biol. Psychiat., 22, 104-7, 1987). In contrast, mouse IFN alpha altered the daily rhythm of activity and food intake in mice. These results agree with the prior findings of species specificity in the physiological effects of IFN. Thus, reports of neurophysiological and behavioral effects of human IFN in rodents should be interpreted with caution.

We have developed an immunocytochemical technique for detecting IFN in tissues which has successfully detected the production of IFN in poly rI-rC induced astrocytes.

This work was supported by MH00621, MH09718.

512.13

SYNTHESIS OF THE HUMAN INTERLEUKIN-1α cDNA AND ITS EXPRESSION IN *E.coli*. **R.A.Ashton, A.Blume, B.Beer and M.Vitek. Dept. of CNS Biology, American Cyanamid Co., Lederle Labs., Pearl River, NY 10965**

Interleukin-1 (IL-1) functions peripherally as a mediator of the acute phase of the immune response. IL-1 can demonstrably evoke responses within the CNS as well, including induction of fever, hypothalamic release of corticotropin releasing factor, and increased slow-wave activity in the sleep EEG. Central effects of IL-1 may be mediated through receptors located throughout the brain (Farrar *et al., J. Immunol.*, 139: 459-463, 1987). In order to study the function and distribution of IL-1 in the CNS, we sought to obtain the IL-1α protein and its corresponding cDNA. While this could be accomplished by screening a cDNA library using conventional hybridization techniques, we elected, instead, to synthesize the cDNA corresponding to the mature and active form of the human IL-1α protein. Twelve oligonucleotides were synthesized, six per strand, annealed together, and ligated. The 468 base pair DNA was then ligated into the prokaryotic expression vector, pEV-vrf1 (Crowl *et al., Gene*, 38: 31-38, 1985) to yield pCLL706.2. DNA sequencing confirmed that the pCLL706.2 sequence exactly matched nucleotide positions 351 to 816 of the IL-1α sequence reported by Gubler *et al. (J. Immunol.*, 136: 2992-2997, 1986). This cDNA was transformed into *E. coli* expressing a temperature sensitive cI repressor protein. Shifting the growth temperature from 30° to 42° induced translation of a 17 kDa protein, the size of the mature IL-1α protein. "Western" blots of this protein react strongly with an anti-IL-1α polyclonal antibody (a generous gift of Dr. R. Chizzonite, Hoffmann LaRoche, Inc.). Finally, lysates of heat shocked cells containing pCLL706.2 stimulated [^{3}H]thymidine uptake into mouse thymocytes about 6000 fold more than control lysates. Based on these data, we conclude that pCLL706.2 directs the synthesis of a biologically active recombinant human IL-1α protein.

512.14

TUMOR NECROSIS FACTOR IMMUNOREACTIVE INNERVATION IN THE MOUSE BRAIN. C.D. Breder and C.B. Saper, Depts. of Pharm. and Physiol. Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637

Tumor necrosis factor α (TNF) is a macrophage derived cytokine that is responsible in part for the cerebral component of the acute phase reaction and chronic response to infection and cancer. TNF, like other cytokines such as interleukin-1 (IL1), is thought to enter the central nervous system through the circumventricular organs (CVOs), and to act on local neurons. However, it has recently been demonstrated that IL1 is found in an intrinsic neuronal system within the brain, and acts on neurons at some distance from the CVOs. We have used an antiserum against recombinant murine TNFα to examine the hypothesis that there might also be a TNF-like immunoreactive (TNFir) neuronal system in the brain. TNFir cell bodies were seen the hypothalamus, primarily in the paraventricular nucleus and in the medial preoptic and lateral hypothalamic areas. TNFir fibers were observed innervating the lateral septum, the bed nucleus of the stria terminalis, the paraventricular nucleus of the thalamus and several regions of the hypothalamus, including the dorsomedial, paraventricular, periventricular and suprachiasmatic nuclei and the lateral hypothalamic area. In the brainstem, TNFir fibers were found to innervate the central gray matter, the parabrachial nucleus and the dorsal vagal complex. Our findings suggest that the TNFir neuronal system in the mouse brain may play a role in the autonomic and endocrine responses that occur during the inflammatory response.

512.15

NPY-POSITIVE NERVE TERMINALS CONTACT LYMPHOCYTES IN THE PERIARTERIOLAR LYMPHATIC SHEATH OF THE RAT SPLENIC WHITE PULP. J.A. Olschowka, S.Y. Felten, D.L. Bellinger, D. Lorton, and D.L. Felten. Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

There is a large body of evidence that sympathetic noradrenergic nerve fibers innervate smooth muscle associated with blood vessels, cardiac muscle, and secretory glands throughout the body. In many instances, neuropeptide Y has been shown to be co-localized within these noradrenergic nerves. Earlier we have demonstrated that noradrenergic nerve fibers, in addition to supplying the smooth muscle of the splenic capsule, trabeculae and blood vessels, also form very tight appositions with lymphocytes of the periarteriolar lymphatic sheath (J. Neurosci. Res. 18:38-48, 1987). The possibility that neuropeptide Y may directly effect lymphocytes by direct apposition of nerve terminals was studied by using electron microscopic immunocytochemistry to localize NPY within the spleen.

Immunocytochemistry was used to demonstrate NPY-like positive profiles in the spleen of the adult Sprague Dawley rat. At the light microscopic level, numerous varicose nerve profiles were observed in the white pulp, particularly surrounding the central arteries and their arteriole branches. At the electron microscopic level, NPY terminals were observed in close proximity to smooth muscle cells of the arteries and directly abutting lymphocytes. There was no intervening cell processes between these presumed T-lymphocytes of the periarteriolar lymphatic sheath and the labeled varicosities. The opposing membranes were smooth, evenly spaced. This association, like that for the noradrenergic system, provides a route by which the autonomic nervous system may directly influence specific immune system effector cells. We are currently working to determine the possible co-localization of NPY within noradrenergic fibers of the spleen.

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512.16

LHRH PARTICIPATES IN NEURO-IMMUNE-ENDOCRINE INTERACTIONS. B. Marchetti*, V. Guarcello*, M.C. Morale* and U. Scapagnini*(SPON: M. Motta). Dept. of Pharmacology, Medical School, University of Catania, 95125 Catania, Italy.

The present paper adresses the question of whether the hypothalamic peptide LHRH might function as a natural link in the bidirectional regulation of neuroendocrine and immune systems function. Indeed, specific binding sites enabling LHRH to act upon the rat thymus gland,have been characterized. Thymic LHRH binding sites undergo important changes associated with marked modifications of the immune response, namely puberty, castration and aging.Detection of rat anti-BSA antibodies of the IgG class following 15 days in the serum of young and old rats immunized with multiple injections of complete Freund adjuvant and BSA confirmed the profound suppres sion of the humoral immune response in aging animals, while LHRH-analog treatment produced a marked increase in anti-BSA antibodies titers. Moreover, the in vitro response of thymocytes from both young and old rats to the mitogen concanavalin A (Con-A) was 7-fold higher in LHRH-treated animals. In addition, LHRH agonists directly potentiate Con-A-stimulated blastogenesis,suggesting that these peptides may also modulate immune system function.

512.17

INTERLEUKIN-2 ENHANCES CHICK AND RAT SYMPATHETIC, BUT NOT SENSORY, NEURITE OUTGROWTH. P.K.Haugen*and P.C.Letourneau, Dept. of Cell Biol. and Neuroanat., Univ. of Minn., Mpls., MN 55455.

An increasing amount of evidence suggests the nervous and immune systems are interdependent. Lymphokine growth factors may represent a link between these two systems. We wished to determine if one such lymphokine, interleukin-2 (IL-2), affects neurons in vitro. To determine if IL-2 affects neurons, sympathetic and sensory neurons from chick and rat were cultured in media with human recombinant IL-2. Ganglia from E9-E11 chicks and neonatal rats were dissected, dissociated in 0.25% trypsin, and plated on dishes coated with laminin. Cells were cultured in serum free media with or without IL-2 (2-200U/ml) and with or without NGF. IL-2 enhanced the number of neurons with neurites in chick sympathetic chain and rat SCG neurons by 200-300% over cultures without any growth factors. Sensory neurons from chick and rat DRGs were not affected by culture in IL-2. Further, sympathetic neurons cultured in IL-2 were 200-300% longer than those cultured without IL-2. In chick, the enhancement of neurite outgrowth and length by IL-2 is nearly the same as that seen with NGF. Cells cultured in NGF and IL-2 together did not show an increase in neurite outgrowth, but did have increased neurite length over cultures containing NGF or IL-2 alone (chick only). Neuron response to IL-2 was dose dependent, with an optimum around 0.2U/ml (0.1 ng/ml or 10 pM) for chick sympathetic neurons and around 2U/ml (1 ng/ml) for rat SCG. Concentrations as low as 10fM were still effective. Cultures which had been preplated to enhance for neurons by removing the more adherent fibroblasts and glia showed the same enhancement of neurite outgrowth by IL-2 as cultures which had not been preplated. This suggests IL-2 acts directly on neurons, and not by stimulating glial cells.

512.18

ANALYSIS OF STRUCTURE RELATED ACTIVITY OF CALCITONIN IN THE IMMUNE SYSTEM: COMPARISON WITH BRAIN. A.E.Panerai, and P.Sacerdote, Dept. Pharmacology, School of Medicine, University of Milan, Italy.

Calcitonin (CT) is a hypocalcemic peptide which possesses a broader range of effects including effects on the Central Nervous System. Recent data suggest that peptides can modulate several immunological functions. We have analyzed the human monocyte chemotaxis activity of different molecular forms of CT, whose activity spectrum in the bone and in the brain are well known. The rank potency order both on the brain and the bone is: eel>CT salmon CT > human CT,while CGRP, derived from an alternative RNA splicing, is the principal form and the more active in the brain. On the contrary, eel CT is the less active on chemotaxis (CI=2 at 10^{-11} M), salmon is only slightly more potent than human CT (CI=3.5 and 3, respectively) and CGRP is devoid of any chemotaxis effect. It is possible to modulate the chemotactic activity of the monocyte CT receptor, in fact monocytes of CT treated patients show an impaired chemotactic response to CT suggesting a down regulation of the receptors. These data suggest a possible specific role of CT in the immune system.

NEURAL CONTROL OF IMMUNE SYSTEM IV

513.1

RAPID DECREASE IN PERIPHERAL IMMUNE RESPONSES FOLLOWING INTRACEREBRAL INFUSION OF INTERLEUKIN-1 (IL-1) OR STIMULATED ENDOGENOUS RELEASE OF IL-1. Syam K. Sundar,* Kyra J. Becker* and Jay M. Weiss (SPON: A. Tadepalli). Duke University Medical Center, Durham, NC 27710.

Interleukin-1 (IL-1) is known to have widespread biological activities. Recent findings indicate that IL-1 activates the hypothalamic-pituitary axis when injected centrally as well as peripherally, and may be a neuromodulator (or neurotransmitter) in brain. The present results show that central administration of human recombinant beta IL-1 caused a rapid (within 15 minutes) and dramatic decrease in peripheral immune responses. Infusion of non-pyrogenic doses of beta IL-1 (dose range 0.05ng to 0.25ng) into the lateral ventricle of rats caused a significant and dose dependent decrease in Natural Killer Cell (NK) activity, responses of lymphocytes to a mitogen, and IL-2 production by activated lymphocytes. In addition, these effects of infused IL-1 could be blocked by central adminstration of a-MSH, a hormone known to block the actions of IL-1 (dose a-MSH=1.0ng). To determine whether release of endogenous IL-1 in brain might also have the same effects as infused IL-1, lipopolysaccharide (LPS), a substance known to provoke IL-1 synthesis and release, was infused into the lateral ventricle (dose LPS=10ng). LPS suppressed immune responses as did IL-1, and these effects of LPS infusion were similarly blocked by central infusion of a-MSH. These results indicate that IL-1 infusion into the central nervous system has direct effects on immune responses and that endogenous release of IL-1 in the CNS has similar effects.

513.2

STRESSFUL CONDITIONS ENHANCE AS WELL AS SUPPRESS CELLULAR IMMUNE RESPONSES. Jay M. Weiss, Syam K. Sundar,*and Kyra J. Becker* Department of Psychiatry, Duke University Medical Center, Durham NC 27710.

Non-chronic stressful conditions have almost always been found to produce immunosuppression in both humans and animals. The present results indicate that, although different intensities of stress produced immunosuppresion, stressful conditions of moderate intensity produced immunoenhancement in rats. In assessing a range of stressor conditions, effects of milder stressors than are usually studied (handling and exposure to a few brief shocks) were examined as well as effects of stronger stressors (2 hours and 19 hours of tail shock). Natural Killer cell (NK) activity, T-cell mitogenesis to Phytohemagglutinin (PHA), interleukin-2 (IL-2) production, and IL-2 receptor expression were measured. As the intensity of the stressor increased, the response of immunological cells was first mildly suppressed (in response to handling), then enhanced (in response to a few brief shocks), and then profoundly suppressed (in response to 19 hours of tail shock). These results demonstrate that the relationship between intensity of the stressor and the effects observed on a variety of measures of cell-mediated immunity is not only complex (or multiphasic) but also include a "zone" in which non-chronic stress enhances various measures of immune function.

513.3

DOMINANCE AND IMMUNITY IN NONHUMAN PRIMATES: SOME PILOT OBSERVATIONS. M. Laudenslager, M. Boccia*, and P. Held*. Behavioral Immunology Lab, University of Colorado Health Sciences Center, Denver, CO 80204.

The concept of dominance has served as an important heuristic tool for the understanding of social relationships in a variety of species. The present pilot study assessed the relationship between dominance ranking in laboratory reared group-housed macaque monkeys and several in vitro measures of the immune response. We found in stable social groups that neither mitogen stimulation nor natural cytotoxicity was related to dominance status. Plasma levels of IgG, C3, and C4 were lowest in the highest ranking monkeys, but remained within the normal range. The introduction of an ecologically relevant challenge may be necessary for the demonstration of modulation of the immune response by social status. We found that the imposition of clumped food resources, as opposed to distributed resources, was associated with reduced cytotoxicity measures in low ranking animals only. All animals, regardless of rank, showed an increase in mitogen responses under clumped feeding resources. High rank may partially buffer against the impact of environmental stressors. (Supported in part by Public Health Service Grants MH37373 and MH44131)

513.4

ALTERED T-LYMPHOCYTE RESPONSIVENESS IN C3H MICE FOLLOWING A NON-AGGRESSIVE TEST FOR DOMINANCE. C-A. Hardy, J. Quay*, R. Ader, and S. Livnat*. Dept of Psychiatry, U. of Rochester Med Ctr., Rochester, NY 14642.

Intermale aggression is a natural form of psychosocial stress that can alter a variety of physiological functions, including immune function. Spontaneous fighting among laboratory mice, often requiring long periods of isolation, pain, or the introduction of trained fighters may be needed. Subsequent fighting behavior often results in wounding which may confound the interpretation of the role of stress in producing immunological changes.

In the present experiment, 6 wk old C3H/HeJ male mice (n=20) were pair-housed (PH) for 4 wks. Individually- (IH)(n=5) and group-housed (GH)(n=5/cage) comparison groups were included. For PH mice, a test trial involved simultaneously placing each mouse head-first into opposite ends of a 10.5 in horizontal tube (1.1 in diameter) so that their snouts touched, requiring one animal to back out to escape. IH and GH mice were tested in the tube individually. All subjects were tested for 14 consecutive days, 1 trial/day. On d15, spleen cells were tested for proliferative responses to mitogens, interleukin-2 (IL-2) production, and natural killer (NK) cell activity.

In PH mice, the same mouse of each pair backed out of the tube on all 14 test days. Of the 10 pairs, 9 of the 10 animals that backed out of the tube appeared dominant in observations of home cage behavior. T-cell proliferative responses to ConA and IL-2 production were increased in the mice that backed out compared to their cagemates and to IH and GH controls. NK activity was not different between groups.

Using this simple, relatively non-aggressive test in PH mice, we found that mice that backed out of our testing tube (i.e. usually the dominant mouse of the pair) had elevated T-lymphocyte responsiveness, which may influence susceptibility to certain disease processes. (Supported by: NIMH Training Grant T32 MH18822, NIMH grant K01 MH00572, and a research grant from RJR Nabisco Co.)

513.5

Social Confrontation in Aggressive Fish (Tilapia) is associated with an Endogenous Opioid System-Mediated Suppression of Immunological Functions- Faisal, M [1,2], Chiappelli, F. [2,3], Ahmed, I [1], Cooper, E. [1] and Weiner, H. [3]. Departments of Anatomy [1] Microbiology and Immunology [2], and Psychiatry and Biobehavioral Sciences [3], University of California at Los Angeles, LA, CA 90024.

The interaction between the neuroendocrine and the immune systems is well-documented (Jankovic et al., 1987). Elegant studies reported that an intermittent footshock in rats leads to naltrexone(NAL)-reversible analgesia and suppression of splenic 'natural killer' cytotoxic activity (Shavit et al., 1985). Social confrontation evokes the massive release of prolactin, ACTH, alpha-MSH and beta-endorphin (END) in intruder and subordinate rats (Smelik, 1987). We previously showed that social confrontation among aggressive fish (e.g., Tilapia) leads to a marked suppression of several immunological parameters, including cytotoxicity and mitogen-stimulated proliferation, in the subordinate fish (Faisal et al., In Press; Cooper et al., In Press). In this study, we show that this immunosuppression is mediated in part by endogenous opioids. NAL (1mg/100g body weight) injected (s.c.) 60 min before social confrontation (5 hrs) prevents the suppression in cytotoxicity (lytic units at 20% lysis) in the dominant (saline: 12.2; NAL: 17.2) and the subordinate fish (saline: 1.7; NAL: 6.6). NAL also modulates the proliferative responses in the subordinate fish to phytohemagglutinin (saline: 33%, NAL: 64%) and concavalin A (saline: 34%, NAL: 65%), but not to lipopolysaccharide derived from Escherichia coli (saline: 27%, NAL: 34%). Isolated pronephric lymphocytes treated with END (10-8M) also show suppressed cytotoxicity and proliferative responses, which are reversible by NAL treatment in vitro. The serum isolated from subordinate fish suppresses the function of the cells obtained from control and dominant fish in a similar pattern.

513.6

PANIC ATTACKS PRODUCE AN INCREASE IN NATURAL KILLER CELL ACTIVITY. T.W. Uhde, M. Geraci*, and R.J. Weber*. BPB, NIMH, and NIDDK, NIH, Bethesda, MD 20892.(spons: D. Matsumoto).

Certain individuals suffer from a syndrome known as panic disorder. In a subgroup of these patients panic attacks can be induced by oral administration of caffeine. Eight patients were examined prior to and following oral caffeine challenge for peripheral blood natural killer(NK) cell activity. An exact temporal correlation was found between psychopathological measures consistent with a diagnosis of panic attacks and intense anxiety and concommitant increases in NK cell activity. Caffeine administered to normal volunteers caused no such change in NK cell activity. A single patient received caffeine on two separate occasions, only one of which produced panic attacks and increases in NK. Changes in NK cell activity were not observed when caffeine administration produced no generalized anxiety or panic attacks. These findings provide direct clinical evidence to support the notion that CNS activity associated with panic disorder, the major features of which are intense anxiety , fear and helplessness, can produce alterations in immune function.

513.7

SELF-ADMINISTRATION OF CYCLOPHOSPHAMIDE BY AUTO-IMMUNE MICE. T. Schachtman*, J. Moynihan*, L. Grota and R. Ader*. Department of Psychiatry, University of Rochester Medical Center, Rochester, NY 14642.

Autoimmune MRL-lpr mice consume more chocolate milk containing cyclophosphamide than congenic +/+ mice that are not autoimmune. The hypothesis is that autoimmune mice are able to determine that the immunosuppression resulting from cyclophosphamide is immunotherapeutic for them, while for +/+ mice immunosuppression is not adaptive. Male lpr mice but not female lpr mice exhibit this behavior when first exposed to drug at 20 weeks of age. We examined several factors that influence consumption of cyclophosphamide focusing on differences between lpr and +/+ females. Diluting chocolate milk reduces palatability and the ability to mask the flavor of cyclophosphamide but does not discriminate lpr and +/+ females. Drinkometers refined measurement of consumption but did not discriminate between female lpr and +/+ mice when first exposed to drug at 20 weeks of age. Both male and female lpr mice consume more cyclophosphamide than +/+ mice when first exposed to drug at 16 and 18 weeks of age. Since the onset of the autoimmune disease occurs several weeks earlier in females than males, these data suggest that the behavioral effect of immunotherapy may depend on the initiation of immunotherapy early in the development of the disease. These data indicate that immune status is able to modify behavior (CNS function) in autoimmune mice.

513.8

CRITICAL PERIODS FOR STRESSOR PROVOKED IMMUNOLOGICAL CHANGES. S. Zalcman*, M. Richter* and H. Anisman. Dept. of Psychology, Carleton University, Ottawa, Ont. Canada K1S 5B6 and Dept. of Microbiology and Immunology, University of Ottawa, Ottawa, Ont. Canada K1H 8M5.

The effects of stressors on the plaque forming cell response and on antibody titers to sheep red blood cells in mice are dependent upon the time of stressor application and the stressor regimen applied. A critical period exists after antigen administration (approximately 72 hr) during which footshock suppressed the immune response. Stressor effects were enhanced in mice that had been exposed to a single stressor session several weeks prior to immunization. Interestingly, however, initial exposure to the aversive stimulation either 24 hr prior to or immediately after antigen administration, prevented the suppression ordinarily elicited by a subsequent stressor. Moreover, compensatory changes were induced by chronic shock, such that the immunosuppression ordinarily elicited by subsequent stressor was eliminated. In fact, under these conditions an immunofacilitation was evident.

513.9

MICROIONTOPHORETIC APPLICATION OF MURAMYL-DIPEPTIDE ALTERS THE DISCHARGE FREQUENCY OF HYPOTHALAMIC AND HIPPOCAMPAL NEURONS. P.M. Dougherty* and N. Dafny (SPON: D. Redburn). Dept. Neurobiol. & Anat., The Univ. of Texas Medical School at Houston, 77225.

Muramyl-dipeptide (MDP) is an endogenous metabolite of gram negative bacterial lipopolysaccharide (endotoxin) with a variety of biologic effects. The present study investigates whether immunological products can elicit a change in the discharge frequency of single neurons in the CNS following local application of MDP within the somatosensory cortex, hippocampus, and the medial basal hypothalamus. The results obtained from a total of 90 cells of forty male Sprague-Dawley rats, demonstrate a direct, stereo-specific effect of MDP upon all three brain regions studied. Among these areas, the hypothalamus was the most responsive (64%), while the cortex was least reactive (47%). The direction of change induced by MDP was most frequently excitatory in all areas although scattered inhibition and biphasic responses were also seen. Since the hippocampus and hypothalamus have previously been shown to also play a key role in the integration of various environmental stimuli into behavioral and physiologic processes, these findings suggest that products derived from immune responses, such as MDP, may act directly in the CNS to coordinate autonomic and endocrine function into the systemic response to disease.

513.10

MURAMYL-DIPEPTIDES ALTER THE NEURONAL FIRING RATE OF THE HYPOTHALAMUS AND HIPPOCAMPUS BUT NOT THE DORSAL RAPHE. N. Dafny and P.M. Dougherty. Dept. Neurobiol. & Anat., The Univ. of Texas Medical School at Houston, 77225.

Muramyl-dipeptide (MDP) is the minimal fragment necessary for the biologic activity induced by gram negative bacterial lipopolysaccharide or endotoxin, and is hypothesized to participate in the mediation of neuro-immune inter-communication. The present study is an investigation of the electrophysiologic activity of single units recorded from freely behaving animals previously implanted with permanent electrodes within the hypothalamus, hippocampus and dorsal raphe prior to and then following incremental systemic (i.p.) dosages of 6-O-Stearoyl MDP. The results obtained from a total of 184 cells of male Sprague-Dawley rats demonstrate that single neurons of the hypothalamus and hippocampus, areas previously shown to play a role in the integration of various environmental stimuli into behavior and physiologic processes, alter their firing in rather site-specific manners. This specificity includes both the sensitivity as well as the time course of responses to MDP. In contrast, other brain regions such as the dorsal raphe showed little effect following MDP administration. These results suggest that MDP may play a role in neuro-immunologic regulatory pathways during the immune response to bacterial infections.

513.11

EVALUATION OF SUPPRESSED LYMPHOCYTE RESPONSIVENESS INDUCED BY SHOCK: ASSESSMENT OF INTERLEUKIN 2 DEPENDENT AND INDEPENDENT PATHWAYS. J. E. Cunnick*, D. T. Lysle*, A. Armfield*, and B. S. Rabin* (SPON. T. Mendelson). Div. of Clin. Immunopath., Dept. of Pathology, Univ. of Pittsburgh, Pittsburgh, PA 15213-3417.

Presentations of 16 signaled foot-shocks is capable of suppressing the spleen lymphocyte mitogenic response to concanavalin A (Con A). This suppression was not due to changes in total leukocytes, or the percentage of T cells, T-helper cells, or T-nonhelper cells as determined by flow cytometry. Although the addition of recombinant IL-2 stimulated the mitogenic response of splenocytes from shocked and control rats, the response of shocked splenocytes remained suppressed in comparison to all controls. Splenocytes from shocked rats produced normal amounts of IL-2 in response to Con A stimulation. Preliminary analysis of IL-2 receptors on the stimulated lymphocytes showed no quantitative differences between shocked and control rats. Splenocytes from shocked rats also demonstrated a suppressed incorporation of 3H-thymidine when stimulated with calcium ionophore A23187. In conclusion, shock-induced suppression of mitogenic responsiveness was not due to changes in lymphocyte subpopulations or IL-2 production. However, a calcium dependent biochemical pathway may be associated with the suppressed T cell responsiveness.

513.12

STRAIN DIFFERENCES IN IMMUNOLOGICAL RESPONSES TO STRESS. J. Irwin, S. Zalcman* and H. Anisman. Depts. of Psychology, Queen's University, Kingston, Ont. K7L 3N6 and Carleton University, Ottawa, Ont. K1S 5B6.

Genetic factors contribute to behavioral and neurochemical consequences of stress exposure. In the present study the influence of such factors on immune reactivity following stress was investigated. Because catecholamines have been implicated in the relationship between stress and immunity, noradrenergic activity was also assessed.

After stress exposure, mice of several inbred strains were sacrificed for determination of splenic Natural Killer (NK) cell cytotoxicity and C.N.S. catecholamine content. NK activity was significantly suppressed at intervals from 30 min to 48 hr following 1 hr of intermittent footshock stress. The time course of the effect as well as the magnitude of the stress-induced suppression varied across inbred strains. For example, a significant reduction of NK was noted in C3H/HeJ mice 24-48 hr after stress, whereas among C57BL/6J mice NK was reduced within 30 mins. of stress offset. Catecholamine alterations engendered by stress also varied across strains. This neurochemical reactivity was apparently more pronounced in those strains which exhibited a larger or more rapid inhibition of NK cell activity after stress.

These data indicate that there are significant strain-specific variations in the response of the immune system to stressors. Furthermore, they provisionally suggest that alterations in catecholamine activity may contribute to the effects of stress on immune function. (Supported by NSERC Grant U0569)

513.13

NEURONAL EXPRESSION OF MHC CLASS I MOLECULES IN RESPONSE TO AXONAL INJURY IS A GENERAL PHENOMENON. M. Schultzberg*, J. Maehlen and K. Kristensson. Dept. of Pathology, Karolinska Institute, Huddinge Hospital, S-141 86 Huddinge, Sweden.

Molecules coded in the Major Histocompatibility Complex (MHC) are absent in normal brain tissue. Such molecules are generally induced by immunological reactions, and this has also been shown in the brain. However, in some peripheral organs, MHC expression may also be controlled by trophic hormones. There is recent evidence that injury of a peripheral nerve triggers MHC class I expression in axotomized motor neurons and this reaction is reversed upon nerve regeneration (Maehlen et al., 1988). The aim of the present study was to investigate whether MHC class I is also induced in nerve cells terminating within the brain, and the nigrostriatal dopamine system was selected. The median forebrain bundle was interrupted either stereotactically or by 6-hydroxydopamine injection (4 ul, 2 ug/ul). The rats were decaptitated 5 and 7 days later and the brains were snap frozen and processed for immunohistochemistry with monoclonal antibodies to class I molecules. Class I-like immunoreactivity was encountered in many neurons in the substantia nigra (SN) ipsilateral to the injury, while the contralateral SN was unstained. These findings suggest that the appearence of MHC class I in injured neurons is a general phenomenon, that may play a role in cellular immunity or other cell-cell interactions in the central nervous system.

513.14

IMPAIRED ANTIBODY PRODUCTION WITH DEFEAT IN RATS. M. Fleshner*, M.L. Laudenslager, L. Simon*, and S.F. Maier. Dept. of Psychology, Univ. of Colorado, Boulder, Co. 80309.

Studies have linked physical stressors with changes in immune function. We examined the effect of a social stressor, defeat due to territorial defense, on antibody production to a specific antigen, keyhole limpet hemocyanin (KLH). Pairs of male rats living undisturbed 3-6 months formed colonies. Test rats were intruders. Every animal was immunized with KLH prior to exposures to territorially defensive colonies. Control animals were placed into colonies but separated from residents by a barrier. Behavioral measures were taken for each colony-intruder interaction. Excluding immunization, the entire procedure was repeated one week later. Blood samples were taken one, two and three weeks following immunization. KLH Ab levels were determined. Experimental animals produced less Ab to KLH than controls. Within the experimental group, total time spent in submissive posture at week one was the best predictor of Ab production. Total bites did not correlate with Ab production. Thus, the physical aspect of defeat (bites) is not critical for suppressed Ab production in defeated rats. Instead the psychological component seems most important. (Supported in part by MH37373 and N0014-85-K-0411 ONR.)

513.15

STRESS AND BRAIN REACTIVE AUTOANTIBODY LEVELS IN MURINE MODELS OF SLE. A. Narendran*, S. Harkins*, S.A. Hoffman. Dept. of Microbiology, ASU, Tempe, AZ 85287.

Psychosocial factors are known to interfere with immune system functioning. Conversely, abnormal immune activity, such as autoimmunity, has been implicated in CNS dysfunction. As part of ongoing research into the nature, function and regulation of pathogenic anti-brain autoantibodies, we have investigated the effect of stress on the production of such molecules.

Animals from 4 autoimmune strains (MRL/l, BXSB, NZB/W and NZB) and 2 non-autoimmune strains (Balb/c and C57BL/6) were divided into two age & sex matched groups per strain. The stressor was based on an animal intrusion and overcrowding model. One group (per strain) was stressed by introducing an equal number of C57BL/6 mice into their cage. This would increase 5 mice in a cage to 10 of two different strains. The control group was left with 5 mice in their cages. Seven days later sera from these animals were assayed for autoantibodies against integral brain membrane proteins using an ELISA. We report the stress induced changes in the anti-brain autoantibody levels in the different strains and discuss the implications of our findings for the effects of stress on autoimmunity.
(Supported by Flinn Foundation Grant # 023-109-062-87.)

513.16

NATURALLY OCCURRING AUTOANTIBODIES IN HUMAN SERUM: REACTIVITY WITH CENTRAL NERVOUS SYSTEM PROTEINS. J.S. Frazier* and D.M. Jacobowitz (Spon: W. Heydorn), Lab. of Clin. Sci., NIMH, Bethesda, MD 20892

Autoimmunity is being recognized as a major component, if not cause, of an increasing number of diseases including those of the CNS. In an effort to identify autoantibodies associated with various neurologic and psychiatric diseases it was discovered that sera from normal individuals contain autoantibodies which react with a number of CNS proteins.

Two-dimensional polyacrylamide gel electrophoresis was performed on 100 µg samples of frozen normal human cortex. Proteins on the resulting gels were Western blotted onto nitrocellulose membranes. Non-specific membrane binding sites were blocked with 3% BSA. The blots were then incubated individually with sera from 20 normal controls at dilutions of 1:10 to 1:1000. Bound autoantibodies were labeled and visualized by the methods of Towbin.

Reactivity against a number of CNS proteins was observed in a large percentage of sera. These proteins included, among others, neuron specific enolase, glial fibrillary acidic protein (GFAP), the ß-subunit of the G protein, serum glutamic oxaloacetic transaminase (SGOT), actin and tubulin. The identity of these proteins was confirmed by comparing the location of the blot-spots with the locations of spots produced with antibodies specific for these proteins. Antibodies against these proteins may be important in the normal and the diseased state.

514.1

FACTOR BINDING TO A CRF-INDUCIBLE ELEMENT OF THE RAT POMC GENE S.R.J. Salton, J.L. Lundblad, S. Dermer, D. Lorang, M. Blum and J.L. Roberts (SPON: R. Fremeau, Jr.). Fishberg Center in Neurobiology, Mount Sinai School of Medicine, NYC, NY, 10029

Expression of the gene encoding proopiomelanocortin (POMC), the precursor of several pituitary peptide hormones including ACTH, ß-endorphin, and ß-lipotropin, has been previously shown to be positively regulated by the hypothalamic peptide CRF, both in vivo and in primary anterior pituitary cultures in vitro. We have found that treatment of the AtT20 mouse pituitary cell line with CRF for 60 minutes increases POMC gene transcription by 2 fold, and after treatment with CRF for 24hrs, a 2-3 fold increase in POMC mRNA levels is observed. By constructing a series of deletion mutants of the rat POMC gene 5' flank fused to a heterologous promoter and chloramphenicol acetyl transferase (CAT) reporter gene, followed by transfection into AtT20 cells, we have identified a fragment within the POMC flank that confers CRF and forskolin inducibility on the TK promoter (-234 to -133). We have identified a factor(s) in heparin-agarose enriched AtT20 whole cell extracts that protects a 30bp region (-214 to -174) on both strands within this fragment using DNase footprinting. Analysis of the region of the rat POMC promoter between -478 and -320, an area which appears partially responsible for the elevated basal activity of the POMC promoter by transient transfection assay, using gel shift studies, DNase footprinting and methylation interference studies, shows several sites protected by factors present in nuclear extracts of AtT20 cells. Studies are currently underway to determine the hormone inducibility and cell/tissue distribution of these DNA-binding factors.

514.2

REGULATION OF GLUCOCORTICOID RECEPTOR GENE EXPRESSION IN THE RAT ANTERIOR PITUITARY. K.E.Sheppard*, D.J.Autelitano*, J.L.Roberts and M.Blum. Fishberg Research Center in Neurobiology , Mt. Sinai Med. Ctr., New York, NY 10029.

It has previously been shown that chronic treatment with dexamethasone (DM) decreased glucocorticoid binding capacity in rat anterior pituitary, however this decrease in binding was not observed after chronic stress. The differences observed between DM treatment and stress may reflect the involvment of other factors in GR regulation in the pituitary.

To further examine GR regulation in the rat anterior pituitary, we have analyzed cytoplasmic mRNA levels after acute and long term CRF treatment, as well as GR gene transcription after acute CRF treatment. For acute studies adult female Sprague-Dawley rats (~ 200g) were injected s.c. with 20µg of r-CRF or vehicle and sacrificed after 30', 60' and 4hrs. In chronic studies, rats were similarly injected twice daily for 7 days.To determine GR gene transcription rate, an *in vitro* nuclear run-on assay was used; cytoplasmic mRNA levels were quantitated in a solution hybridization/S1 nuclease protection assay using an antisense RNA probe.

A 2-4 fold increase in GR gene transcription was observed at 30 and 60 min after CRF administration, but had returned to control levels after 4hrs. Cytoplasmic GR mRNA levels were significantly above control at 4hrs. after CRF treatment; in contrast, chronic treatment (7 days) failed to show any change. To determine if the CRF response is a direct effect, or is mediated through ACTH stimulated release of glucocorticoids we are currently examining the effects of CRF and glucocorticoids on GR mRNA levels and GR gene transcription in primary cultures of rat anterior pituitary, as well as in AtT20 cells.

514.3

DEVELOPMENT OF BASAL AND REGULATED SECRETION IN THE INTERMEDIATE LOBE OF THE FETAL AND NEONATAL RAT PITUITARY GLAND. D.I. Lugo and I.E. Pintar. Department of Anatomy and Cell Biology, Columbia University P&S, NYC, NY. 10032.

Proopiomelanocortin (POMC) producing cells comprise nearly 100% of the adult intermediate lobe hormone producing cells. Secretion by these cells is primarily under negative regulation by dopamine. Although the POMC-derived peptide a-MSH has been detected in plasma of fetal rats and lambs, no study thus far has directly examined the secretory capabilities of fetal melanotrophs. Here we have utilized the reverse hemolytic plaque assay to assess at the single cell level basal and regulated release by melanotrophs at fetal and early post-natal ages. Only basal secretion was detected at the earliest ages examined (e17.5). CRH (10^{-8}M.)-stimulated secretion was first observed at e19.5 and continued through post-natal ages; incubation with CRH (10^{-7}M.) during this time period increased both the plaque size and the percentage of melanotrophs stimulated to secrete. Dexamethasone (10^{-6}M.) inhibition of CRH (10^{-7}M.) stimulation was detected from e19.5 to p2. At p3 dexamethasone no longer inhibited melanotroph secretion although inhibition of CRH-stimulated release in p3 corticotrophs was readily detected. The dopamine agonist ergocryptine (10^{-6}M.) inhibited secretion at the earliest ages studied (e17.5); this effect persisted throughout all ages examined (e17.5-p3). These results suggest that melanotrophs appear to undergo a maturation process in which they are first non-responsive to CRH (e17.5), next possess both functional CRH and steroid receptors (e19.5) and finally undergo loss or uncoupling of steroid receptors (p3). The loss of steroid-induced inhibition (p3) is closely coupled with the arrival of catecholaminergic input into the neurointermediate lobe (p2). However, the early response of melanotrophs to dopaminergic agonists, which can be detected seven days prior to arrival of catecholaminergic fibers into the neurointermediate lobe, appears to be an intrinsic feature of these cells that is never present in corticotrophs. Supported by HD-18592.

514.4

RECEPTOR REGULATION OF POMC GENE EXPRESSION IN PRIMARY CULTURES OF RAT INTERMEDIATE LOBE LOBULES. M.S.Rinaudo*, B.M.Chronwall, W.R.Millington, J.F.Bishop and D.R.Gehlert. ETB-NINCDS, N.I.H., Bethesda, Maryland 20892.

The intermediate lobe (IL) of the rat pituitary is primarily melanotrophs which are organized into clusters of cells called lobules. Previous culture systems using dispersed IL cells may not represent cellular behavior in vivo accurately since earlier data from our laboratory have shown that melanotrophs growing in monolayer exhibit a substantially higher proliferation rate compared to the IL in vivo. To study both the regulation of proopiomelanocortin (POMC) biosynthesis and IL proliferation, we have recently developed a primary culture system of rat IL cells using mechanically dispersed intact IL lobules. The effects of CRF, GABA and quipazine ($5HT_2$ agonist) on the synthesis of POMC mRNA, release of immunoreactive β-endorphin (iβEND) and cell proliferation were studied utilizing this model.

CRF increased the amount of iβ-END secreted at both a 10^{-9} and 10^{-7} M dose. Both concentrations of CRF augmented the relative levels of POMC mRNA although the increase was statistically significant only with the higher dose. Quipazine also increased the levels of POMC mRNA significantly at both the low (10^{-8} M) and high (10^{-6}M) doses. Total iβ-END secreted into the media was significantly increased only with the higher dose of the two compounds. No alteration in proliferation was seen with either compound. Inclusion of GABA in the media at concentrations up to 10 µM as well as muscimol and baclofen failed to produce a detectable alteration in the levels of POMC mRNA, iβ-END secretion and proliferation rate.

In conclusion, CRF and $5HT_2$ agonists not only increase the release of POMC derived-peptides from mechanically dispersed IL lobules but also increase the levels of POMC mRNA. On the other hand, GABAmimetics did not appear to affect mRNA levels nor iβ-END secretion.

514.5

INTERACTIONS OF OPIOID PEPTIDES, NALOXONE AND DOPAMINE ON POMC PEPTIDE RELEASE FROM RAT NEUROINTERMEDIATE LOBES. L. Gutierrez*, A. Samora*, M. Williams* and L.C. Saland. (SPON: E. Uhlenhuth). Dept. of Anatomy, Univ. of New Mexico Sch. Med, Albuquerque, NM 87131.

Release of POMC (α-MSH and β-endorphin) peptides from the pituitary pars intermedia is regulated by both neurotransmitters and neuropeptides. Dopamine (DA), an inhibitor, suppressed the corticotropin-releasing factor (CRF)-induced stimulation of peptide secretion in vitro (Saland et al, '87, Soc. Neurosci. Abst. 13: 418, and Neuropeptides, '88, in press). Here, rat neurointermediate lobes (NILS) were incubated for up to 120 minutes in GIBCO media containing glucose, glutamine, 0.1mM bacitracin, 0.1mM ascorbic acid, and 10 µM pargyline. D-ala-met-enkephalinamide (DALA, 10^{-6}M) or Sandoz (SAN) enkephalin peptide (10^{-6}M) were added, with or without DA (10^{-6}M) or naloxone (NAL, 10^{-6}). DALA or SAN plus DA transiently increased POMC release above levels in the presence of DA alone at some, but not all time points. NAL alone reduced α-MSH release, while addition of NAL to peptides plus DA induced POMC release above control levels in several incubations. POMC peptides were measured by radioimmunoassay (RIA). Immune staining of tissues for POMC peptides and EM cytology of NILS correlated with relative amounts of peptides released. Effects of opioid peptides or NAL may be mediated via interaction with DA, since dopamine-2, but not opioid receptors, are found on intermedia cells. Supported by NIH NS 21256 and RR 08139 (LCS).

514.6

EFFECTS OF cAMP AND CORTICOTROPIN-RELEASING FACTOR (CRF) ON PROTEIN PHOSPHORYLATION AND ACTH RELEASE IN THE ANTERIOR PITUITARY. J.C. Pryor*, S.T. Cain, and C.B. Nemeroff (SPON: E.W. Busse). Depts. of Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.

Phosphoproteins appear to be involved in the secretion of anterior pituitary hormones. A main focus of our laboratory has been CRF, which stimulates the release of adenocorticotropin (ACTH) from the anterior pituitary. Because CRF receptors are coupled to adenylate cyclase, we began investigating the role of cyclic 3'-5'adenosine monophosphate (cAMP)-dependent protein phosphorylation in CRF stimulated ACTH secretion. We have already established in an in vitro system that cAMP stimulates the phosphorylation of at least 4 different proteins in anterior pituitary homogenates obtained from rats. These have approximate molecular weights by SDS-gel electrophoresis of 93K, 63K, 48K and 43K. The reaction appears to be dependent on the concentration of cAMP with 1 µM cAMP stimulating phosphorylation of only the 93K protein, while maximal stimulation was achieved with 50 µM cAMP. To better simulate in vivo conditions, we are currently investigating CRF and cAMP stimulation of ACTH release and protein phosphorylation in a cell culture system. Adult, male rats were decapitated and anterior pituitaries were harvested. Enzymatic disassociation was used to obtain individual cells and they were cultured in multiwell culture plates. The cells were stimulated with CRF (0-10 nM) for 3.5 hr. ACTH released into the media was measured by RIA. The cells were then homogenized and aliquots phosphorylated for 60 seconds in the presence of 10 µM ATP containing [γ^{32}P-ATP] and either cAMP or vehicle. The proteins were separated by electrophoresis on SDS-polyacrylamide gels, and autoradiograms of the phosphoproteins were prepared from the dried gels. Results of studies investigating the association between CRF stimulation, cAMP formation, ACTH release and protein phosphorylation will be presented. (Supported by NIMH MH-42088 and the United Way Fund.)

514.7

IN VITRO MODELLING OF STRESS-INDUCED ACTH SECRETION. Michael Boyle*, Paul Plotsky, Gayle Yamamoto* and Wylie Vale, The Salk Institute, La Jolla, CA, 92037

ACTH secretion by the anterior pituitary is stimulated by several humoral and neural pathways, including corticotropin-releasing factor (CRF), vasopressin (AVP) and catecholamines. In the present studies, we have examined ACTH release from rat pituitary tissue using a range of concentrations found in hypophsial-portal blood of rats. At levels approximating endogenous molar ratios, these secretagogues were tested for their ability, alone and in combination, to stimulate ACTH release. To more closely mimic the response found *in vivo*, freshly removed rat anterior pituitaries were used in a superfusion system. At concentrations above 300 nM CRF elicited a maximal 6 fold increase in the release of ACTH. The EC50 for the CRF induced ACTH release was 12 nM. At concentrations above 300 nM AVP ellicited a maximal 1.7 fold increase in the release of ACTH. The EC50 for the AVP induced ACTH release was 7 nM. At concentrations above 1 μM the catecholamines epinephrine (E) and norepinephrine (NE) elicited a maximal 2.6 fold increase in the relase of ACTH. The EC50s for E and NE were 17 nM and 32 nM respectively. The EC50s for each of these compounds is well within the ranges found in hypothalamic-pituitary portal blood. The combination of CRF and AVP is additive across the full physiologic range of the secretagogues with a maximal 7.3 fold increase of ACTH release. Mimicing the increased CRF and AVP concentrations observed in hypophysial-portal plasma during hemorrhage, 150 pM and 800 pM respectively, a similar 2-3 fold elevation of ACTH is seen. The combination of CRF and E is less than additive with a maximal 7.8 fold increase in ACTH release. This is the first report of effects of endogenous portal levels of secretagogues on ACTH secretion *in vitro*, and indicates the validity of this particular preparation as a model of stress-induced ACTH release.

514.8

EXPRESSION OF BIOLOGICALLY ACTIVE RAT CORTICOTROPIN RELEASING FACTOR (CRF) IN A TRANSFECTED MEDULLARY THYROID CARCINOMA (MTC) CELL LINE. G. Hammer*, V. Fairchild*, K. Sevarino* and M. Low*. (SPON: R. Ventimiglia). Neuroscience Graduate Program, Tufts U. School of Medicine and Div. of Molecular Medicine, New England Medical Center, Boston, MA 02111.

CRF is a neuropeptide that acts as both a hypophysiotropic regulator of ACTH secretion and a mediator of central autonomic responses to stress. Studies of the biosynthesis of CRF have been limited by the lack of a suitable tissue culture system. To provide such a system, we have engineered clonal cell lines that express high levels of rat CRF. W2 cells, a transplantable rat MTC cell line (provided by B. A. Roos, Univ. of Wash., Seattle, WA), were cotransfected with a CRF cDNA (provided by R. Thompson, Oregon Health Sciences Univ., Portland, OR) driven by a cytomegalovirus immediate early promoter/enhancer and a neomycin resistance-expressing plasmid driven by an RSV promoter. These cells were chosen as the host cells because of their high concentration of secretory granules and their ability to process and amidate neuropeptides. Media from G418-resistant clones were screened by radioimmunoassay to detect CRF expression. The transfected, but not the wild type cells, secreted assayable CRF. To assess biological activity of the immunoreactive CRF, dispersed primary pituitary cultures were treated with conditioned media from the 6 clones secreting the highest concentration of CRF (1- 9 nM). ACTH release from the pituitary cells was stimulated identically by treatment with either synthetic CRF-amide or transfected W2 cell conditioned media. In conclusion, we have made stably transfected cell lines that express rat CRF. The secreted immunoreactive CRF is biologically active suggesting that the pro-CRF produced is correctly processed and amidated. Studies are in progress to characterize the biosynthesis of CRF in these cells further and to use them, via surgical implantation, as an ectopic source of CRF in transkaryotic rats.

514.9

MOLECULAR MECHANISMS CONTROLLING POMC GENE EXPRESSION. Neil Margolis, Kyriaki Thermos and Terry Reisine, Dept. of Pharmacology, Univ. of Pennsylvania, Phila., PA 19104

The control of POMC gene expression is an important event in the body's response to stress. The molecular events controlling POMC gene expression are currently unknown. Since several ligands are known to both increase POMC mRNA levels in the anterior pituitary and activate protein kinases, it is possible that phosphorylation is a key event in the control of POMC gene expression. We have examined the effects of corticotropin releasing factor (CRF), which is known to activate cAMP-dependent protein kinase, on phosphorylation of nuclear proteins. Using AtT-20 cells, a mouse anterior pituitary cell line, we have found at least three nuclear proteins which are phosphorylated in response to CRF (10^{-7} M). These proteins, which have been visualized using two-dimensional gel electrophoresis and autoradiography, have molecular weights of 45,49, and 56 kilodaltons and pI values between 5.9 and 6.4. Protein phosphorylation has been shown to be enhanced within five minutes of CRF treatment. It is possible that these proteins interact with upstream elements of the POMC gene to cause an increase in DNA transcription. Work is currently being done to examine the effects of phorbol esters and calcium ionophores, which increase POMC mRNA levels and can activate distinct protein kinases, on phosphorylation of nuclear proteins. Supported by NIH grant DK37404 and American Heart Association grant-in-aid.

514.10

LOCALIZATION OF POMC mRNA, GLUCOCORTICOID RECEPTOR mRNA AND CRF RECEPTORS IN HUMAN PITUITARIES. I.F. Lopez*, A. Mansour, H. Akil, S. Burke*, M. Palkovits*, M. Arato*, M.K.-H. Schafer* and S.J. Watson. (SPON: S. Berent). Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109 and National Institute For Nervous and Mental Diseases, Budapest, Hungary.

Human hypothalamic-pituitary axis regulation has been the focus of extensive investigation. A knowledge of how different components of this system are related anatomically can give clues to their interaction and co-regulation. We have studied the localization of pro-opiomelanocortin (POMC) and glucocorticoid receptor (GR) mRNA in post-mortem human pituitaries using *in situ* hybridization analysis. We have also looked at the localization of Corticotropin Releasing Hormone (CRH) receptors by autoradiography in the same tissue. To control for total number of cells, *in situ* hybridization with p1B15 (Sutcliffe, J.G., et al., Nature, 308:237, 1984) a cDNA clone of a ubiquitous rat mRNA was carried out in adjacent sections.

P1B15 containing cells were distributed homogenously in the pituitaries examined. In contrast, groups of POMC cells were localized in clusters. Cells containing CRH receptors were also found in clusters and for the most part were localized in the same area as the POMC cells. GR containing cells were distributed through most of the pituitary but some areas of increased density were observed to coincide at times with areas of increased POMC density.

514.11

α-ANF[1-28] BUT NOT α-ANF[5-28] INHIBITS CRF-STIMULATED ACTH SECRETION FROM CULTURED ANTERIOR PITUITARY CELLS. M.S. King* and A.J. Baertschi. Neuroscience Program and Department of Physiology, University of Virginia, Charlottesville, VA 22908.

The effectiveness of atrial natriuretic factors (ANF) as inhibitors of ACTH secretion was examined in dispersed rat anterior pituitary cells that had been in culture for 4 days. Nineteen experiments were conducted with 24-well plates using 4 wells/combination of peptides. α-ANF[1-28] significantly inhibited ACTH release stimulated by 1nM CRF. At the most effective concentrations of 10 to 100pM, α-ANF[1-28] inhibited ACTH release by 45% ($p<.001$: determined by Kruskal-Wallis ANOVA). This effect was manifested after 3 hours, but not .5 or 1 hour, of incubation suggesting that ACTH synthesis may have been reduced by α-ANF[1-28]. Conversely, at concentrations of 10 to 10,000pM, α-ANF[5-28] had no effect on ACTH secretion after .5, 1 and 3 hours. These results suggest that: 1) an intact N-terminal sequence of the ANF peptide, 2) extremely low concentrations of α-ANF[1-28] and 3) a 3 hour incubation are required for inhibition of ACTH. These requirements may explain the failure of previous experiments to demonstrate inhibition of ACTH by ANF. Thus, α-ANF[1-28] may be a physiological inhibitor of ACTH secretion. (Supported by University Technology Corporation and the Virginia Center for Innovative Technology).

514.12

THYMOSIN MODULATES BETA-ENDORPHIN SECRETION BY CORTOCOTROPHIC TUMOR CELLS. J. Michel, J.M. Farah. G.D. Searle & Co., CNS Research, St. Louis, MO 63198.

Hormones of the thymus gland have been associated with control of the hypothalamic-pituitary adrenal axis partly because the bovine thymic preparation, thymosin fraction 5 (TSN-5), was shown to increase secretion of ACTH both in vivo and in vitro. We found that TSN-5 increased secretion of immunoreactive beta-endorphin (iβ-E), from AtT-20 corticotropic tumor cells and its effects on hormone release were additive with those of corticotropin-releasing factor (CRF) (Farah et al, 1987, J Neurosci Res 18:140). We now find that TSN-5 evoked iβ-E release is additive not only with CRF but also with forskolin and phorbal myristate acetate. Since TSN-5 had no effect on basal intracellular cAMP and reduced forskolin-stimulated cAMP accumulation, the corticotropic constituent(s) of TSN-5 probably act through alternate cellular mechanisms to promote iβ-E secretion. The effects of TSN-5 on hormone release coupled to arachidonate metabolism and intracellular calcium mobilization are under investigation.

Our thanks to Dr. Allan Goldstein for TSN-5.

514.13

A PRACTICAL RADIOIMMUNOASSAY FOR PLASMA CORTICOTROPIN - RELEASING FACTOR. J.C. Ritchie, P.K. Liu*, G. Bissette, and L.H. Jennes. Department of Psychiatry, Duke Univ., Durham, NC 27710 and Department of Anatomy, Wright State Univ. Sch. Med., Dayton, OH 45435

We will describe an RIA for plasma Corticotropin - Releasing Factor (CRF). The primary antiserum for this assay was produced in rabbits against rat/human CRF coupled to hemocyanin via glutaraldehyde. The antiserum is used at a dilution of 1 to 30,000. Radiolabelled CRF is prepared by chloramine T iodination of Tyr^0-CRF and is purified by HPLC. Samples for assay are drawn into cold EDTA coated plastic syringes containing 0.1 ml of an enzyme killer solution per mL of whole blood. Plasma is extracted using two Spice Pak Cartridges (Analtech, Inc. Newark, DE) hooked in tandem. Standard curves are prepared in CRF-free plasma and extracted as samples. Recovery averages 80% (by trace-recovery studies). The assay incorporates delayed trace addition and second antibody technology to improve sensitivity. The minimal detectable quantity for the assay is 2.5 pg/mL and the 80, 50, and 20% binding points average 7, 80, 900 pg/mL respectively. No cross reactivity is detectable with ovine CRF up to 1 ug/mL. Cross reactivity with other neuropeptides is negligible.

Data concerning baseline concentrations, physiologic and physiochemical validations of the assay will also be presented.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION III

515.1

PLASMA ACTH IN THE RAT DEMONSTRATES THREE DISTINCT RHYTHMS WITHIN 24 HOURS. M. Carnes, S.J. Lent*, J. Feyzi* and D.K. Hazel*. Wm. S. Middleton Veterans Hospital and Dept. of Medicine, University of Wisconsin, Madison, WI 53705

ACTH secretion by the pituitary is rhythmic and episodic, as reflected by fluctuations in plasma concentrations of ACTH. The present work was designed to further characterize the patterns of ACTH secretion occurring simultaneously within a 24-hour period in the rat. Blood sample collection protocols with sampling intervals of 2 minutes, 15 minutes, and 4 hours were used in awake, chronically cannulated rats. Plasma samples were assayed for ir-ACTH and resultant data were analyzed for significant pulsatile secretory episodes using the PULSAR program after determination of appropriate constants. Three distinct patterns of ACTH secretion were demonstrated within a 24-hour period. In addition to the circadian variation with peak plasma ACTH levels occurring one hour before lights-out, plasma ACTH exhibited episodic ultradian variation of two types: 11-19 pulses in 24 hours, which we have called the "larger ultradian" pulses, and shorter episodic bursts occurring approximately 3 times per hour, which we have called "micro-pulses."

515.2

NEUROTENSIN ACTIVATES TUBEROINFUNDIBULAR DOPAMINE NEURONS AND INCREASES SERUM CORTICOSTERONE CONCENTRATIONS. G.A. Gudelsky, S. Berry* and H.Y. Meltzer. Case Western Reserve University, Cleveland, OH 44106.

In view of the evidence for co-localization of neurotensin (NT) and dopamine in some neurons within the arcuate nucleus-median eminence, we have examined the effects of NT on tuberoinfundibular dopamine (TIDA) neurons, as well as on mesolimbic and nigrostriatal neurons in the rat. The activity of these DA neurons was estimated from the accumulation of DOPA in the median eminence 30 min after decarboxylase inhibition with NSD 1015 (100 mg/kg, i.p.) or from the concentrations of DOPAC in the median eminence, n. accumbens and striatum. The icv administration of NT (20 ug) significantly increased the accumulation of DOPA and DOPAC concentrations in the median eminence 1-8 hrs after its administration. NT (5 and 20 ug) also significantly increased DOPAC concentrations in the n. accumbens, but the peptide was without effect in the striatum. Serum corticosterone concentrations in rats treated with NT (1-20 ug) were 5-7 times those in vehicle-treated rats. An analogue of NT, [D-Trp^{11}]-NT (0.5 ug, icv) also significantly increased DOPAC concentrations in the median eminence and serum corticosterone concentrations. It is concluded that NT acutely increases the activity of TIDA and mesolimbic dopaminergic neurons and increases the secretion of ACTH. Supported by MH 41684 and MH 42868.

515.3

EFFECT OF ALPHA-MELANOCYTE STIMULATING HORMONE (αMSH) ON TUBEROHYPOPHYSIAL, TUBEROINFUNDIBULAR, AND NIGROSTRIATAL DOPAMINERGIC NEURONAL ACTIVITY. S.E. Lindley*, K.J. Lookingland and K.E. Moore, Dept. of Pharmacology/Toxicol., Mich. State Univ., E. Lansing, MI 48824

The purpose of this study was to determine if αMSH feedsback to regulate its own secretion by altering the activity of tuberohypophysial dopaminergic (THDA) neurons terminating in the intermediate lobe (IL) of the pituitary. Accordingly, the effect of intracerebroventricular (icv) administration of α MSH was examined on THDA neuronal activity. For comparison, the effect of αMSH was also examined on the activity of tuberoinfundibular dopaminergic (TIDA) neurons terminating in the median eminence and nigrostriatal dopaminergic (NSDA) neurons terminating in the striatum. The activity of DA neurons was estimated using biochemical techniques. Administration of αMSH (20 µg, icv) to male Long-Evans rats did not alter: 1) the concentration of dihydroxyphenylacetic acid (DOPAC), 2) the rate of DA turnover, 3) the rate of DA synthesis in the IL or striatum. These results indicate that exogenously administered αMSH does not alter THDA or NSDA neuronal activity. In the same animals it was found that α MSH increased: 1) the concentration of DOPAC, 2) the rate of DA turnover, 3) the rate of DA synthesis in the median eminence. α MSH also decreased circulating concentrations of prolactin. These results indicate that αMSH increases the activity of TIDA neurons terminating in the median eminence, thereby decreasing the secretion of prolactin. (Supported by NIH grant NS 15911)

515.4

EVIDENCE THAT ACTIVATION OF THE HPAA BY SINGLE-DOSE ADMINISTRATION OF ETHANOL IS NOT MEDIATED BY MEDIAN EMINENCE AVP OR ADRENAL CATECHOLAMINES. A.B.THIAGARAJAN* R.L.ESKAY* (Spon: S.KATZ). LCS/NIAAA, National Institutes of Health, Bldg 10, Room 3C-218, Bethesda, MD 20892.

Single-dose ethanol (Et) administration activates the hypothalamic-pituitary-adrenal axis (HPAA) as monitored by enhanced levels of adrenocorticotropic (ACTH) hormone, glucocorticoids (Corticosterone, Cs) and adrenomedullary-derived epinephrine (Epi); however, an understanding of the precise mechanism or site of ethanol's activation of the HPAA remains incomplete. In order to determine if median eminence (Me)-derived vasopressin (AVP) or adrenal-derived Epi are significant mediators of Et-induced activation of the HPAA, Et was infused (12% solution, 4gm/Kg/body wt.) via an indwelling gastric cannula into male rats which had previously (two weeks) been adrenal demedullated or pretreated with AVP antiserum. Assessment of the ability of the AVP antiserum to neutralize endogenous AVP was demonstrated in that this antiserum reduced ether-enhanced ACTH plasma levels by 40%; however the AVP antiserum did not attenuate ethanol-induced ACTH or Cs plasma levels. In addition, demedullation did not lessen activation of the HPAA by Et. The results of this study suggest that single-dose administration of Et via intragastic cannula does not involve recruitment of Me AVP or adrenal Epi as partial mediators of the Et-induced activation of the HPAA.

515.5

REDUCTION OF ACTH-LI IN PLASMA FOLLOWING I.V. INJECTION OF DELTA SLEEP-INDUCING PEPTIDE IN MAN. A. Bjartell, R. Ekman*, S. Bergquist, E. Widerlöv*. Dept. of Psychiatry and Neurochemistry, Univ. of Lund, POB 638, S-220 06 Lund, Sweden.

Recent studies have indicated in involvement of Delta sleep-inducing peptide (DSIP) in the regulation of the hypothalamic-pituitary adrenal (HPA) axis. We have now examined how DSIP affects the plasma levels of adrenocorticotropin-like immunoreactivity (ACTH-LI), cortisol and some other neuropeptides related to the HPA axis in man. The present study is a double blindcross-over challenge test in which eleven healthy male volunteers (age 25-29) achieved a single dose of synthetic DSIP (25 nmol/kg b.w.) intravenously (iv) during 4 min. An equal volume of saline served as control. The plasma conc. of DSIP increased approximately three times (mean)at 5 min after DSIP inj. and was then rapidly normalized. ACTH-LI in plasma decreased in nine out of eleven individuals and the mean conc. of ACTH-LI in plasma was significantly reduced at 5-180 min after DSIP inj. The controls showed an increased ACTH-LI level. No differences in plasma cortisol were noted between the groups. Urinary analyses of stress parameters like cortisol and monoamine metabolites did not reveal any differences either. In conclusion: A single dose of DSIP iv significantly reduces the plasma conc. of ACTH-LI in man and may thus be yet another factor involved in the regulation of ACTH secretion in man.

515.6

REEVALUATION OF NOREPINEPHRINE (NE) EFFECTS ON THE EXTRACELLULAR SINGLE UNIT ACTIVITY OF PARAVENTRICULAR (PVN) NEUROSECRETORY NEURONS. R.L. Moss and Y.I. Kim. Dept. of Physiology, UT Southwestern Med. Ctr., Dallas, Texas 75235.

In an attempt to provide a possible explanation for the conflicting reports concerning the role of NE in the control of the activity of PVN neurosecretory neurons projecting to the posterior pituitary, the effects of both electrical stimulation of the A1 noradrenergic region (the major noradrenergic input to PVN neurons) and iontophoretically applied NE on the activity of PVN neurosecretory neurons were tested in urethane-anesthetized male rats. Of 11 phasically active (P) and 84 non-phasically active (N-P) neurons studied, 5 P and 46 N-P neurons exhibited an excitatory orthodromic response (OD+) to A1 region stimulation while only 3 N-P neurons exhibited an inhibitory orthodromic response. The remaining neurons showed no response. Iontophoretically applied NE was excitatory as was A1 region stimulation in 4 of 4 P neurons tested and in 16 of 32 N-P neurons tested. However, in 9 N-P neurons exhibiting OD+, iontophoresed NE was inhibitory. Three of these 9 neurons were identified antidromically as projecting to the A1 region as well as to the posterior pituitary. The inhibitory effect of iontophoresed NE on the neurons exhibiting OD+ was selectively abolished by the co-iontophoresed alpha blocker, phentolamine, but not by the beta blocker, timolol, in 4 of 4 cases tested. This includes 2 cases where the neurons were identified as projecting to both the posterior pituitary and the A1 region.

In general, the present results agree with the postulation of an excitatory A1 noradrenergic input to PVN neurosecretory neurons. In addition, the results suggest the presence of an alpha-receptor-mediated, inhibitory noradrenergic input other than A1 to a subpopulation of PVN neurons. This may explain, at least in part, the earlier observation that NE exerts an inhibitory effect on the activity of neurosecretory neurons. Supported by HD09988-V.

515.7

THYROTROPIN-RELEASING FACTOR (TRF) EFFECTS ON PITUITARY ACTH RELEASE AND AUTONOMIC FUNCTION. M.R. Brown[1], C. Rivier[2] and T.S. Gray[3]. [1]Depts. of Medicine and Surgery, Univ. of Calif., San Diego Medical Center, San Diego, CA 92103; [2]The Salk Institute, La Jolla, CA 92037; [3]Dept. of Anatomy, Loyola Medical Center, Maywood, IL 60153.

TRF, similar to corticotropin-releasing factor (CRF), acts within the brain to increase plasma levels of catecholamines (CA), and mean arterial pressure (MAP) and heart rate (HR). The goal of these studies was twofold: 1) to determine if TRF, similar to CRF, given intracerebroventricularly (icv), would increase ACTH secretion; and 2) to determine if the CRF-like actions of TRF could be attenuated using a CRF-receptor antagonist, α-hel CRF 9-41. Experiments were performed in awake animals equipped with chronic right atrial, femoral artery, or icv cannulae. Plasma levels of ACTH and CAs, and HR and MAP were measured using established methods. TRF (800 pmols, 2.6 nmols) or CRF (2 nmols) given icv produced increases in plasma concentrations of ACTH and CA, and of MAP and HR. TRF-induced elevation of plasma ACTH levels was dose related and did not occur following iv administration of TRF. α-hel CRF 9-41, at a dose (12 nmols) that inhibited CRF-induced (2 nmols given icv) elevation of plasma levels of ACTH and CAs, and MAP and HR, did not modify TRF's actions. Since TRF does not act directly on the pituitary or via CRF release to stimulate ACTH secretion, alternative mechanisms must exist, e.g. vasopressin, CAs, or unidentified factors, to mediate the observed ACTH release.

515.8

DIURNAL INFLUENCE ON THE RELATIONSHIP BETWEEN BIOACTIVE AND IMMUNOREACTIVE ACTH IN RATS. P. Miller*, C. Cornell*, C. T. Graeber*, and W. C. Engeland (SPON: C. F. Allen-Rowlands). Sect of Neurobiology/Dept. of Surgery, Brown Univ./R.I. Hosp., Providence, RI 02902

The pituitary-adrenal system in rats is characterized by a diurnal rhythm that affects resting plasma corticosterone (B) and B responses to stress. A dissociation between changes in plasma ACTH immunoreactivity (IR) and B has suggested that extra-ACTH factors contribute to diurnal variations in B. To assess the possibility that plasma ACTH-IR does not reflect ACTH bioactivity (BIO), diurnal changes in ACTH-BIO and ACTH-IR were compared. Groups of rats(n=5-12/group) were decapitated and blood was collected in the morning (AM) and in the evening (PM) without stress, 10 minutes post ether stress, and 7 days post bilateral adrenalectomy (ADX). ACTH-BIO was assayed in plasma extracts using collagenase-dispersed rat adrenal cells; B responses were assayed by HPLC-UV. Plasma ACTH-IR was assayed by RIA using antisera directed against ACTH 1-24 and plasma B was assayed by RIA. Resting ACTH-BIO increased diurnally in the absence of a change in ACTH-IR. The ACTH-IR/BIO ratio was 8.0 in the AM and 3.6 in the PM. The AM-PM change in ACTH-BIO was two-fold(5.8±0.4 vs. 11.8±2.6pg/ml), whereas the change in B was 20-fold(6±1.3 vs. 136.1±18.4ng/ml). Ether stress resulted in increases in ACTH-BIO and ACTH-IR that were not different diurnally. However, the ACTH-IR/BIO ratio decreased to 1.5 in the AM and to 1.8 in the PM. There was no AM-PM difference in B responses to ether determined by subtracting resting B. ADX resulted in increases in ACTH-BIO and ACTH-IR that were not different diurnally. After ADX the ACTH-IR/BIO ratio decreased to 1.2 in the AM and to 1.9 in the PM. These data show that diurnal changes in ACTH-BIO occur without changes in ACTH-IR. Since the amplitude of the ACTH-BIO rhythm is less than that of the B rhythm, the B response cannot be explained totally by changes in ACTH-BIO. The ACTH-IR/BIO ratio decreases in response to stress and ADX, suggesting that ACTH-IR is a more accurate index of ACTH-BIO during hypothalamic-pituitary activation. Supported in part by NIH grant DK38951.

515.9

ENDOCRINE RESPONSE TO PHYSOSTIGMINE IN ALZHEIMER'S. E. R. Peskind*, M. A. Raskind*, R. C. Veith* and D. M. Dorsa. (SPON: A. Khan). GRECC, VA Medical Ctr., Seattle,WA 98108

Cholinergic neurons in basal forebrain degenerate in Alzheimer's Disease (AD). Both arginine vasopressin (AVP) and corticotropin releasing factor-containing neurosecretory cells are innervated by cholinergic neurons, some of which probably originate in the basal forebrain. Epinephrine (EPI) release is also mediated by a CNS cholinergic mechanism. To assess CNS cholinergic regulation in AD, we measured plasma AVP, β-endorphin (βE), and EPI responses to cholinergic challenge elicited by the cholinesterase inhibitor physostigmine (0.0125 mg/kg IV) in 12 men with AD and compared responses to 12 age-matched normal men.

Physostigmine promptly increased plasma AVP (10 fold), βE (2-3 fold) and EPI (3 fold) in elderly controls. In contrast, AD patients showed attenuated responses to physostigmine. Differences were most pronounced when control and AD patients who experienced nausea (n=2 and 6, respectively) were excluded. Expressed as area under response curves, AD patient AVP (2±1.2 pg/ml/min) and βE (5±1.5 pg/ml/min) were significantly ($p<.02$) less than those of controls (14±5 and 28±4 pg/ml/min, respectively). AD EPI (10±3 pg/ml/min) tended to be lower than that of controls (60±27 pg/ml/min; $p<0.1$).

We conclude that the cholinergic deterioration of AD also influences CNS regulation of neuroendocrine systems.

515.10

PASSIVE AND ACTIVE MEMBRANE PROPERTIES OF ARCUATE NUCLEUS (ARC) NEURONS AND EFFECTS OF OPIOID PEPTIDES. M.D. Loose* and M.J. Kelly, Dept. of Physiology, OHSU, Portland, OR.

Anatomical data have indicated that the rat ARC has a plethora of cell types that contain either dopamine, amino acids or any of numerous peptides. Since the electrophysiological characteristics of ARC neurons have not been described in similar detail we identified the passive and active membrane characteristics of these cells and examined the effects opioid peptides had on them. Intracellular recordings were made from coronal, 400-500 μm thick slices prepared from proestrous rats using established methods (Brain Res. 345:264, 1985). ARC neurons exhibited stable RMPs (-55 to -71 mV), spike amplitudes (53-89 mV) of short duration (0.9 -1.6 ms), high input resistances (100-400 mΩ) and variable time constants (5.0 - 22.8 ms). During 400ms depolarizing pulses 90% of these cells fired continuously whereas, 10% fired a maximum of 3-7 spikes and then remained silent. A majority of neurons exhibited inward rectification. 10% of the cells had a prolonged (> 200 ms) AHP, and 25% had a low threshold spike which was not blocked by TTX (1-3 μm). DAGO (1 μm), an opioid agonist, affected 55% of the cells tested, causing membrane hyperpolarization (4-12 mV) and decreases in depolarizing PSPs. Therefore, subsets of ARC neurons can be distinguished by their intrinsic electrophysiological properties. Furthermore, several of these subsets respond to opioid peptides. (Supported by DA 05158, HD 07133 and HD 00718).

515.11

AMYGDALA DIRECTLY INNERVATES PARVOCELLULAR PARAVENTRICULAR HYPOTHALAMIC CRF, VASOPRESSIN AND OXYTOCIN CONTAINING CELLS. D.J. Magnuson,* and T.S. Gray. (Spon: G.G. Celesia) Dept. Anatomy, Loyola Univ. Med. Sch., Maywood, IL 60153

Phaseolus vulgaris leucoagluttin lectin anterograde tracer (PHA-L) and immunocytochemistry were used to examine whether amygdala cells directly innervated CRF, vasopressin and/or oxytocin immunoreactive cells within the paraventricular hypothalamic nucleus. Iontophoretic injections of PHA-L were placed within the central or medial nuclei of the amygdala of anesthetized 150-250g Long-Evans rats. Two weeks later animals were overdosed with sodium pentobarbital and their brains were fixed through vascular perfusion. Amygdaloid terminals were demonstrated via PHA-L antibodies and immunocytochemistry using a brown DAB reaction. Paraventricular hypothalamic cell bodies were visualized via CRF, vasopressin or oxytocin antibodies and immunocytochemistry using a glucose oxidase-nitro blue tetrazolium reaction. The medial amygdaloid nucleus innervated oxytocin and vasopressin immunoreactive neurons in rostral regions of the paraventricular nucleus. The central amygdaloid nucleus innervated the medial and lateral parvocellular regions of the caudal paraventricular nucleus. Central amygdaloid terminals innervated CRF, vasopressin and oxytocin immunoreactive cells. The present results sugggest that the amygdala can directly influence parvocellular paraventricular nucleus neurons and therefore affect the neuroendocrine output of these cells. (Supported by ONR N00014-88-K-0010).

515.12

BEHAVIORAL AND NEUROENDOCRINE CORRELATES OF DIETHYL ETHER EXPOSURE IN THE MOUSE John R. Glowa Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Diethyl ether has been used extensively as an neuroendocrine stressor, yet little is known of either its behavioral effects or the concentrations over which they may occur. Adult male NIH mice and Sprague-Dawley rats were exposed to a full range of concentrations of ether (1000-30000 ppm) and effects on operant behavior and neuroendocrine response were compared. When operant responding was maintained under FI 60 sec schedules of milk presentation, 30 min or shorter exposures to concentrations of diethyl ether less than 10000 ppm were without behavioral effect, 10000 ppm produced large increases (up to 300%) in responding, and higher concentrations abolished responding (although not necessarily through anesthetic action). Exposure to a similar range of concentrations of ether elevated ACTH and corticosterone in both mice and rats, from baseline levels of 18.2 pg/ml ACTH and 78.44 ng/ml corticosterone to levels in excess of 310.5 pg/ml ACTH (1700% of control) with 5 min exposure w/o altering corticosterone levels, whereas with 30 min exposures corticosterone levels increased 700%.Increaases in neuroendocrine measures generaly preceded those seen behaviorally, across doses. The imidazobenzodiazepine, Ro 15-4513 (ethyl-8-azido-5,6-dihydro-5-meth-6-oxo-4H-imidazo (1,5-a)(1,4) benzodiazepine-3-carboxylate), previously shown to block both in vivo and in vitro effects of ethyl alcohol, decreased FI responding in mice in the present studies, but only at doses of 10-30 mg/kg. Ro 15-4513 elevated ACTH and corticosterone levels in a dose-dependent manner, with increases in both neuroendocrine measures occurring at doses of Ro 15-4513 (3 mg/kg) that were without behavioral effect alone. However, behaviorally inactive doses of Ro 15-4513 were unable to antagonize the rate-increasing effects of diethyl ether.

515.13

SEX DIFFERENCES IN ANTERIOR PITUITARY POMC mRNA AND SECRETION PATTERNS. J. Kotun*, H.-L. Lin*, and H. Akil (SPON: J. Greden). Mental Health Research Institute and Department of Psychiatry, University of Michigan Medical School, Ann Arbor, MI 48109.

The hypothalamic-pituitary adrenal axis and the hypothalamic pituitary gonadal axis are thought to interact at multiple levels. While differences have been described between male and female rats at the level of plasma glucocorticoids, little is known about the differential regulation of POMC in the pituitary corticotrophs across sexes. The hypothalamic POMC system has been more extensively studied with regard to gonadal interactions. In addition, there is a suggestion that estrogen receptors may negatively regulate POMC gene expression in vitro. However, it is not yet clear if such regulation occurs in the anterior pituitary. We have compared male and female rats both basally (across the estrous cycle) and following acute swim stress. Consistent with earlier work the female rats showed, at rest, a significant elevation of corticosterone over male levels. Paradoxically, the females exhibited lower plasma ACTH throughout the estrous cycle. Acute swim stress induced comparable release of ACTH and steroids in both sexes. Anterior pituitary ACTH and β-endorphin content did not differ between males and females or across the estrous cycle; however POMC mRNA in females was significantly lower than that of males. It appears that the elevated circulating steroid levels in female rats may have resulted in a decreased steady state level of POMC mRNA and POMC product release into plasma, without altering the level of peptide stores in the gland. Yet, the female rats maintain their ability to respond to stress challenges. The role of sex steroids in this differential regulation and possible difference at the adrenal and hypothalamic levels require further investigation.

LEARNING AND MEMORY: HUMAN BRAIN

516.1

NEUROPSYCHOLOGICAL CORRELATES OF BILATERAL FRONTAL LOBE LESIONS IN HUMANS. S.W. Anderson, H. Damasio, D. Tranel, and A.R. Damasio. Div. of Behavioral Neurology & Cognitive Neuroscience, The University of Iowa College of Medicine.

The study of the effects of frontal damage in humans has been hampered by the rarity of nonpsychiatric patients with focal lesions to these areas. Here we present comprehensive neuroanatomical and neuropsychological analyses on 5 patients who sustained bilateral frontal lesions as adults. Criteria for subject selection included: 1) stability of chronic lesions caused by vascular event or surgical ablation, 2) lack of damage to nonfrontal cortices, and 3) normal premorbid social and cognitive function. The neuroanatomical study was based on magnetic resonance scans analyzed with a standard procedure. The neuropsychological study included standardized assessment of a broad range of cognitive functions and interviews with the families. All subjects had involvement of orbital and lower mesial frontal cortices bilaterally, but the dorsolateral regions were preserved; 2 had lesions which extended into basal forebrain. All subjects had personality changes which included disturbances of social conduct, decision-making, planning, motivation, and affect. Intellect, language, and perceptual abilities were intact in all, but awareness of the personality disturbances was impaired. Memory for objects and events was defective only in the 2 subjects that also had basal forebrain damage. Bilateral orbito-mesial lesions in humans are thus strongly associated with cognitive and behavioral defects largely specific to the social domain.

516.2

IMPAIRED AUTONOMIC RESPONSES TO EMOTIONAL AND SOCIAL STIMULI IN PATIENTS WITH BILATERAL ORBITAL DAMAGE AND ACQUIRED SOCIOPATHY. D. Tranel, A.R. Damasio, & H. Damasio. Div. of Behavioral Neurology & Cognitive Neuroscience, Univ. of Iowa College of Medicine, Iowa City, Iowa.

The impaired social behavior of patients with bilateral orbital lesions, can be seen as a domain-specific amnesia in which defective activation of learned somatic states plays a key role (Damasio & Tranel, 1988). Our theory predicts impaired autonomic responses to nonverbal "emotional/social" stimuli in these patients. We studied the skin conductance responses (SCRs) of 4 frontal lobe patients and 4 controls to stimuli with either high (target) or low (nontarget) emotional valence. Subjects viewed the stimuli under either a PASSIVE (no response) or ACTIVE (verbal description) response condition. The controls produced significantly larger amplitude SCRs to target stimuli in both conditions. The frontal patients, however, demonstrated a striking dissociation: in the PASSIVE condition, SCRs to targets were severely defective and not different from nontarget responses; in the ACTIVE condition, SCRs were normal (high-amplitude responses to the target, but not the nontarget, stimuli). E.g., patient EVR produced an average SCR of .031 μS for target pictures under PASSIVE viewing conditions, and an average of .218 μS in the ACTIVE condition. These findings parallel the situation outside the laboratory, where EVR is able to behave adequately only when stimulus configurations are presented in verbal form in a modified, non-real-time base.

516.3

DOMAIN-SPECIFIC AMNESIA FOR SOCIAL KNOWLEDGE.
A.R. Damasio & D. Tranel. Division of Behavioral Neurology & Cognitive Neuroscience, University of Iowa College of Medicine, Iowa City, Iowa.

Patients with bilateral lesions in orbital and lower mesial frontal cortices develop changes in social behavior that include inadequate decision making, planning, and conduct. Such patients are unaware of their predicament. The primary cognitive and neural mechanisms for these remarkable changes remain enigmatic and polemical. Based on extensive neuropsychological and psychophysiological experiments in 5 subjects, we propose that the defects (1) stem from impaired coactivation of an appropriate range of memoranda relative to the complex activities, properties, and events that characterize social interactions, and (2) are manifest when "social knowledge" configurations are presented in real-time through nonverbal channels. Prominent among the missing memoranda are somatic states, including those regulated by the autonomic nervous system. The new theory postulates that the reactivation of internal somatic states that co-occurred with pertinent representations of external events, is a necessary step for a comprehensive range of interwoven associations to become available. However, verbal stimuli can bypass this weak link and generate appropriate evocations. In this light, the "acquired sociopathy" of frontal lobe patients can be seen as a domain-specific disorder of generic memory, i.e., a categorical amnesia for social knowledge that precludes activations necessary for behavioral guidance.

516.4

ISOLATED IMPAIRMENT OF RETRIEVAL AND LEARNING OF REFERENCE LEXICON FOLLOWING LEFT ANTEROTEMPORAL DAMAGE.
H. Damasio, D. Tranel, A.R. Damasio. Div. of Behavioral Neurology & Cognitive Neuroscience, University of Iowa College of Medicine, Iowa City, Iowa.

Four patients with combined damage to mesial and nonmesial left anterotemporal structures (entorhinal cortex/hippocampus/amygdala and anterolateral neocortices) maintain accurate nonverbal records of past experiences, and acquire accurate new nonverbal records. By contrast, they have a severe defect in the retrieval and acquisition of the specific verbal tags that denote those experiences. The dissociation is revealed by experiments requiring (a) naming of entities in different domains, (b) learning of new visuo-verbal relationships, and (c) retrieval and new learning of nonverbal entities and relations. The subjects invariably recognize and accurately describe all stimuli and events that they fail to name specifically, and their ability to access and utilize functors, and to generate correct grammatical structure, is intact. Higher-order association cortices and limbic structures in the left anterotemporal region, thus play a key role in the learning and rapid retrieval of verbal markers in the entity domain but not in the relational domain.

516.5

ANATOMIC CORRELATES OF RETROGRADE AMNESIA.
P.J. Eslinger and L. Cermak*. Cognitive Neuroscience Lab, Brown University, Providence, Rhode Island 02903 and Memory Disorders Research Center, Boston VAMC, Boston, Massachusetts 02130

Studies of patients with amnesia have identified a number of structures and interconnecting pathways which are necessary for the learning and retention of new experiences. These neural units include the medial temporal region, anterior/medial diencephalon, basal forebrain, retrosplenial cortex and interconnecting pathways such as the fornix, mammillo-thalamic tract and amygdalofugal pathway. Patients with damage to these and surrounding structures may also exhibit an extended retrograde amnesia beyond the 1-3 years prior to cerebral injury. We report here the results of neuroimaging in patient SS, who developed a severe anterograde and retrograde amnesia following encephalitis 17 years ago at the age of 38 (Neuropsychologia, Vol 21, 1983, 213-234). In addition to bilateral medial temporal lobe damage and possible damage to the posterior ventromedial frontal lobe (structures which have ben associated with predominantly anterograde amnesia), SS sustained cortical damage to the insula bilaterally, to the temporal poles bilaterally, and to the left anterolateral temporal region. By comparison, patient DRB (Arch Neurol 42, 1985, 252-9) has a more severe retrograde amnesia and a highly similar but more extensive pattern of damage.

We conclude that in a neural model of memory, extended retrograde amnesia is related to damage in cortical structures of the insula and/or anterolateral temporal lobes.

516.6

A POSITRON EMISSION TOMOGRAPHIC STUDY OF ANATOMICAL STRUCTURES IN THE HUMAN BRAIN PARTICIPATING IN LEARNING, RECALL AND RECOGNITION OF COLORED PATTERNS.
P.E. Roland, L. Widén and S. Stone-Elander. Dept of Clinical Neurophysiology, Karolinska Hospital and Institute S-104 01 Stockholm, Sweden.

The regional cerebral blood flow (rCBF) was measured with 11C-labelled CH3F in ten young normal volunteers during four conditions lasting 60 s each: rest, visual learning, recall and recognition. The subjects learned ten colored geometrical patterns which contained no other information than shape and color. The color contrast and luminance was balanced. During recall, the subjects, with eyes closed, recalled the appearance of each color-pattern in the same order as originally shown. During recognition the learned patterns were mixed with other similar patterns. Visual learning increased the rCBF in several prefrontal areas, orbitofrontal cortex, insula, hippocampal regions, neostriatum, thalamus, the calcarine cortex, peri-calcarine cortex, and the visual association areas in the posterior superior parietal cortex and precuneus. Recognition of the color-patterns increased rCBF in the same structures. The purely intrinsic recall of the color-patterns did not change the rCBF in the calcarine, peri-calcarine and orbital cortex; the increase of rCBF in posterior thalamus was especially marked, apart from this the structures which increased rCBF were same as during learning and recognition.

516.7

MATERIAL-SPECIFIC MEMORY DEFICITS AFTER UNILATERAL TEMPORAL NEOCORTICECTOMY. (SPON: C. Bushnell).
T. Burke* and J.R.M. Nolan*. Univ. Coll. Dublin, Belfield, Dublin 4, Ireland.

Two studies were carried out that examined aspects of memory in patients who had undergone a unilateral temporal neocorticectomy for the relief of intractable epilepsy in the Richmond Institute, Dublin. In Experiment 1, a forced-choice recognition-memory paradigm was used to assess memory for: (a) abstract words, (b) concrete words, (c) pictures of common objects and (d) abstract geometric designs. The left temporal-lobe group (N=10) was impaired on the verbal materials and the right temporal-lobe group (N=10) was impaired on the abstract designs. These results are congruent with those observed following temporal lobectomy. Experiment 2 examined the effect of eliminating the value of a verbal label on memory for pictures of common objects. The results revealed an impairment in the right temporal-lobe group. The results of these two experiments extend, to the operation of temporal neocorticectomy, the observation of a double dissociation between the effects of left- and right-temporal lobectomy on memory and, thus, demonstrate a temporal neocortical contribution to human memory processes.

516.8

REDUCTION OF PROACTIVE INTERFERENCE FACILITATES ENDURING IMPLICIT RECALL IN KORSAKOFF AMNESICS.
T.W.Parker and A.R.Dobbs* Dept. of Psychology, Camrose Lutheran College, Camrose, Alberta, T4V 2R3.

Korsakoff amnesics and controls were presented with riddles and tested for implicit and explicit recall of these riddles at 10 minutes, 24 hours and 30 days following the presentation phase. Proactive interference (PI) arising from self-generated responses was eliminated for half the subjects by providing them with the answer immediately after presenting the riddle. In contrast the remaining subjects were encouraged to generate their own response to the riddle and then were given the correct answer. A dissociation between implicit and explicit recall was found such that amnesics for whom PI was minimized demonstrated normal levels of implicit recall for up to thirty days, although no explicit recall of the riddles occurred. This demonstration of normal recall of paired-associates is most likely due to the semantic richness of the stimuli and the automatic elaboration which is thought to take place. The results are discussed in terms of the process of restructuring which facilitates subsequent recall of stimuli which promote an "aha" experience.

516.9

NORMAL IMPROVEMENT IN MENTAL ROTATION SKILL IN GLOBAL AMNESIA. L.M. Parsons*, J.D.E. Gabrieli, J. Yucaitis*, & S. Corkin. Department of Brain & Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139. (SPON: Ken Nakayama).

Patient H.M., despite severe anterograde amnesia following bilateral medial temporal-lobe resection, has the learning capacity to substantially improve a skill for mental rotation. He and healthy, age-matched control subjects made verbal responses in judging whether a non-upright letter was in its normal or mirror-reversed form. The stimuli were viewed at each of 12 orientations, 30 degrees apart. On three successive days, subjects performed two sessions, an hour apart, each with 72 trials. As with control subjects, the initially moderate slope of H.M.'s reaction-time/orientation function declined to nearly zero, and his overall mean reaction time and errors decreased. These results suggest that declines of such RT function slopes are due to an increase in the rate of mentally rotating the stimulus to upright and not due to memorizing its form at the tested orientations. Prior reports of preserved perceptual learning have been limited to stimuli viewed at one orientation, and analysis of the conditions producing the preserved learning reported here may reveal the relation between long-term memory structures and operations used to discriminate disoriented stimuli. Our findings are consistent with the view that there is a dissociation in global amnesia between skill learning and fact learning. In 1987, we reported that HM showed no such improvement when this task required a button press response, a result apparently due to his difficulties with a forced-choice motor response.

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516.10

DISSOCIATION BETWEEN TWO KINDS OF PRIMING IN GLOBAL AMNESIA AND ALZHEIMER'S DISEASE. M.M. Keane, J.D.E. Gabrieli, M.M. Kjelgaard*, J.H. Growdon, and S. Corkin. Department of Brain & Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139, & Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

This study shows that dissociable cognitive and neural mechanisms underlie performance on two priming tasks, stem completion and Gollin incomplete pictures. Twenty mildly or moderately demented patients with Alzheimer's disease (AD) and 4 patients with global amnesias (AMN) due to bilateral limbic-diencephalic lesions performed these two priming tasks. Consistent with prior findings on the stem-completion task, the AD group was impaired relative to the AMN group, which performed normally. In contrast, the AD and AMN groups performed similarly on the Gollin Incomplete-Pictures Test. Even though both groups were significantly impaired relative to normal subjects, they still showed significant and equivalent facilitation at a 1-hour delay. These results establish a dissociation between two priming tasks in AD and suggest that separable mechanisms, distinct from fact learning, underlie the two kinds of performance.

Supported by AG06605-02, RR00088, and AG05134.

516.11

PRIMING IN THE AMNESIC PATIENT H.M.: NEW FINDINGS AND A THEORY OF INTACT AND IMPAIRED PRIMING IN PATIENTS WITH MEMORY DISORDERS. J.D.E. Gabrieli and M.M. Keane. Department of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139, & Department of Psychology, Harvard University, Cambridge, MA 02138.

We report new examples of preserved priming in the globally amnesic patient H.M., and present a theoretical interpretation of intact and impaired priming in amnesia and in Alzheimer's disease (AD). We propose that repetition priming on a visual task takes three different forms. (1) Pre-representational priming depends upon perceptual learning mechanisms that facilitate access to representations (letters, words, objects); such mechanisms are spared in amnesia and in AD. (2) Inter-representational priming depends upon learning mechanisms that guide semantic and lexical access to representations in long-term memory; these mechanisms are spared in amnesia but are impaired in mild AD. (3) Post-representational priming depends upon mechanisms that record the outcome of operations on a repre- sentation: such priming is impaired in amnesia and AD. The observed dissociations among the three forms of priming suggest that they have separable neural bases. Dissociations among forms of priming may reveal how distinct neural systems enhance the efficiency of access to representations irrespective of subsequent operations performed upon those representations.

516.12

LONGITUDINAL ANALYSIS OF THE DYSEXECUTIVE SYNDROME IN ALZHEIMER'S DISEASE. J.T. Becker, Alz. Dis. Res. Ctr., University of Pittsburgh, Pittsburgh, PA 15213 USA

The memory disorder in patients with Alzheimer's Disease (AD) may be described as the combination of an amnesic syndrome and a dysexecutive syndrome (Becker, Neuro. Abs, 1987). The performance of a cohort of 122 AD patients and 93 normal controls was analyzed on four tests of delayed recall and new learning, and three tests related to central executive function. Patients were identified as having either a "focal" dysexecutive syndrome (n=3) or an amnesic syndrome (n=4) based on the difference between scores on standardized composite variables.

One year later, 76 AD patients and 87 controls were re-evaluated on the same battery of tests. Additional patients were found to have dysexecutive (n=2) and amnesic (n=1) syndromes. Furthermore, the seven patients who had "focal" patterns of presentation at baseline were now classified as "non-focal".

These data replicate and extend the previous findings. First, more patients were found who met the criteria for focal abnormalities of memory impairment. This adds strength to the hypothesis that AD patients suffer from a loss of Central Executive System function as described in Baddeley's model of Working Memory. Furthermore, the findings that the focal syndromes are not static but can change with time suggests that they represent the relative sparing of a particular neuropsychological function in the face of a progressive dementia.

THE AGING PROCESS II

517.1

SUBSTANCE P AND SOMATOSTATIN COEXIST WITHIN SENILE PLAQUES OF PATIENTS WITH ALZHEIMER'S DISEASE. D. M. Armstrong, W. Benzing, J. Evans*, and L. Hansen*. Dept. of Neurosci., University of California, San Diego, La Jolla, CA 92093.

In recent years we and others have identified several neuropeptide and neurotransmitter-related substances within senile plaques of patients with Alzheimer's disease. At present, it is unclear whether a senile plaque can be identified according to a single transmitter or peptide substance or alternatively whether a plaque contains multiple substances.

In the present study we employed a highly sensitive dual-immunolabeling procedure and demonstrated that substance P- and somatostatin-immunoreactive profiles coexist within senile plaques of patients with Alzheimer's disease. Coexistence of somatostatin and substance P immunoreactivity within the same plaque was observed in the hippocampus and amygdala but not in the neocortex, although the latter region contained plaques within which somatostatin and substance P existed alone. Regardless of the region, the labeled processes were usually enlarged and within the peripheral (i.e., the neuritic) portion of the plaque.

Supported by NIH grants AG05344 and AG05386.

517.2

GLUCOSE-6-PHOSPHATASE ACTIVITY IN HIPPOCAMPAL NEURONS. K. Cullen-Dockstader* and E. Fifkova (SPON: H. Alpern). Dept. of Psychology, University of Colorado, Boulder, Colorado 80309.

Glucose-6-phosphatase (G6Pase) is intimately involved in the mechanism of Ca^{2+} transport into the endoplasmic reticulum (ER). G6Pase in the ER hydrolyzes the glucose-6-phosphate (G6P) whereby it liberates phosphate ions. These are used to capture the actively transported Ca^{2+} into the ER. The G6Pase activity may be cytochemically detected by lead ions. We have, therefore, adapted the Brodwell-Cataldo method (J. Histochem. Cytochem., 31:1077) to be used in the hippocampus. These experiments have shown G6Pase activity in the smooth and rough ER and in the nuclear envelope. Basket cells, the inhibitory interneurons of the dentate fascia and hippocampus, show a considerably higher activity than the dentate granule cells or hippocampal neurons. Also, neurons of the hilus are intensely labeled. This method is an indicator of differential metabolic activities, and it could be useful to detect possible changes in the G6Pase activity associated with senescence. The ultimate goal of these experiments is to demonstrate the extent to which G6Pase activity may be affected by aging and thus account for the decreased Ca^{2+} sequestering capacity of the SER observed in aged rats (Fifkova and Cullen-Dockstader, Brain Res., 376:357).

Supported by the Institute on Aging, grant #AG04804-03.

517.3

AGING AFFECTS THE SIZE OF VESICLES IN THE PERFORANT PATH TERMINALS IN THE DENTATE MOLECULAR LAYER. E. Fifkova and K. Cullen-Dockstader*. Dept. of Psychology, University of Colorado, Boulder, Colorado 80309.

Aging brings about an impairment of a number of neuronal functions, out of which impairment of synaptic transmission is the most critical. Previously we have shown that migration of synaptic vesicles in the perforant path terminals towards the active synaptic site is affected with age as significantly fewer vesicles were found there at resting conditions. The obvious next step was to study the size (cross sectional area) of these vesicles. Rats (Fischer 344) ages 3, 9, 24, and 30 months old were used. Five animals per age group were prepared for electron microscopy. Vesicles were measured in axon terminals of the perforant path in the dentate molecular layer. Perforant path terminals were identified as those contacting dendritic spines. The vesicle cross sectional area was computed from the largest diameter and the diameter perpendicular to it, using an IBM PC-based three-dimensional reconstruction program (Kinnamon et al., Proc. 44th EMSA Meeting, 1986, p. 876). In these preliminary experiments, we have measured 250 terminals per age group. A significant decrease of the vesicle cross sectional area was observed by the 24th month as compared to the 3rd and 9th months. Since the majority of synaptic vesicles is generated by recycling, the present data could be interpreted as a failure of this process during aging. Such a change could bring about an impairment of the synaptic transmission, the severity of which would be enhanced by the decreased number of vesicles transported to the active site (Fifkova and Cullen-Dockstader, Soc. Neurosci. Abstr., 12:271) and by the loss of some axon terminals in the dentate molecular layer (Geinisman et al., Brain Res., 134:541; Hoff et al., J. Comp. Neurol., 205:246). Consonant with this conclusion is the age-related decrease in amplitude of the perforant path presynaptic potentials while their activation threshold remained unchanged (Barnes and McNaughton, J. Physiol., 309:473). Thus, the present data show presynaptic alteration of the perforant path in the dentate molecular layer that could contribute to the decreased synaptic efficacy observed in this region with aging.

Supported by National Institute on Aging grant #AG04804-03.

517.4

AGE-ASSOCIATED MORPHOMETRIC CHANGES IN MONKEY PREFRONTAL CORTEX. M.D. Applegate*, R.G. Struble, C.A. Fleischman*, L.C. Cork*, and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182.

Performance on behavioral tasks dependent upon the integrity of the prefrontal cortex (e.g., direct-delayed response) [Bachevalier & Mishkin, Behav. Brain Res., 20:249, 1986] is impaired commonly in aged monkeys. To examine possible components of neuronal substrates of this impairment, we conducted a morphometric study of prefrontal cortices in young and aged monkeys. Paraffin-embedded, cresyl violet-stained tissue sections (5 μm) from lateral walls of the principal sulci of 14 monkeys (Macaca mulatta, 4-31 years of age) were analyzed for density and size of cells using the Loats image analysis system. Significant age-associated reductions in neuronal size were demonstrated ($r = -0.6759$; $p = 0.008$), but no changes were noted in the density of neurons ($r = 0.2553$; ns). In contrast, the density of glia was increased significantly with age ($r = 0.6008$; $p = 0.023$). The decline in neuronal size occurred primarily in layers II-IV, and the depths of layers III and IV decreased with age. This age-associated pattern of reduced neuronal size and cortical thickness, increased density of glia, and no change in density of neurons parallels results of studies of midfrontal cortices of aged humans [Terry et al., Ann. Neurol. 21:530, 1987]. These changes, among others, may contribute to behavioral deficits that occur in aged monkeys.

517.5

AGE AND NUCLEAR PORE COMPLEX DENSITY IN CA1 PYRAMIDAL CELLS IN THE RAT. A. Topple, E. Fifkova, K. Cullen-Dockstader* and G. Smith*. Dept. Psychology, University of Colorado, Boulder, Colorado 80309.

Studies on the aging hippocampal formation have revealed ultrastructural and physiological alterations that may underlie a number of functional deficits associated with aging. One such change is the reduced density of nuclear pore complexes (NPCs) in the granule cells of the dentate fascia which occurred in the absence of any change in the nuclear envelope perimeter (Fifkova et al., Exp. Neurol., 95, 755, 1987). This decrease is thought to reflect a decrease in the metabolic activity of these cells. We report here our preliminary findings on the density of these pores in a different area of the hippocampal formation, the CA1 pyramidal cells. Ten male Fischer 344 rats aged 3, 9, 24, and 30 months (2 animals per age) were prepared for electron microscopy and were photographed on a JEM-100C electron microscope. There were no differences in the density of pores across ages in the CA1 pyramidal cells. There was, however, a 17.8% increase in the nuclear envelope perimeter with increasing age (which did not reach significance). Such an increase may have masked any change in NPC density. These data suggest that there may be age-related regional differences within the one structure for the same parameter. However, more animals are required before definitive statements can be made regarding the effects of age on NPC density in this area.

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517.6

LOW CALORIE-HIGH FIBER DIET AND AGING: EFFECT ON BRAIN LIPIDS. M.T. Tacconi, L. Lligona, M. Soldati, N Pitsikas, S.Algeri and M.Salmona. Istituto di Ricerche Farmacologiche "Mario Negri, Milano, Italy.

Food intake restriction increases longevity and delays the occurrence of age-related physiological deterioration.We studied whether brain membrane lipids and microviscosity (parameters known to be modified by age) were affected by a diet low in calories. Male rats (Charles River Italy, Calco, Italy) were fed *ad libitum* with standard diet or with a low caloric diet (LCD) in which 50% of lipids and 35% of carbohydrates have been replaced by fibers. The diet was started at weanling. Body weight, food consumption, date and possible cause of natural death were recorded. At 4, 15 and 32 months of age rats were killed, brain dissected and cortex used for analysis. Rats which received the LCD were leaner by 15-30% than their age paired controls. Survival at 32 months of age was 25% in LCD versus 10% in control rats. LCD rats showed the same age-related increase in cholesterol content as control, but total PLs (differently than in control rats) significantly increased, resulting in a reduction of Chol/PL ratios. Similary, Sph/PC ratios were reduced in LCD fed rats in respect to controls. Membrane microviscosity (which increased with age, as expected, in rats fed standard diet) was not affected significantly by LCD. Polyunsaturated FA in PLs (mainly in PE and PS) were lower in rats fed LCD for 32 months than in controls of the same age In conclusion feeding rats with a low calorie-high fiber diet in brain counteracts the age-related increase in Chol/PL and Sph/PC ratios, being not able, however, to inhibit the reduction in fluidity observed with age. Since polyunsatured FA age-related reduction in LCD rats was greater than in controls it is possible to speculate that LCD may produce a partial EFA deficiency, which worsens the age-related decrease in FA desaturases, thus counteracting the positive effect on fluidity of PLs increase.

517.7

REGIONAL CHANGES WITH AGE OF CALPAINS IN RAT BRAIN. A. Kenessey*, M. Banay-Schwartz* and A. Lajtha. (SPON: W. Sacks). Ctr. for Neurochem., N.S. Kline Inst. Ward's Island, NY, NY 10035.

Low (CANP I)- and high (CANP II)-calcium-requiring neutral proteinases (calpains) and their specific endogenous inhibitor (calpastatin) constitute an intracellular regulatory system that plays an important role in the metabolism of cytoskeletal proteins. Differences between the CANP activities in neonatal and adult brain regions have been reported; however, no data are available about their properties and activities in the senescent brain. We measured CANP activities in six brain regions (cortex, cerebellum, striatum, pons-medulla, hypothalamus, and hippocampus) from young adult (3-month-old) and aged (24-month-old) rats, using ^{14}C-methylated casein and brain protein substrates (desmin, actin, tubulin, and neurofilament preparations). Calpastatin was separated from the two enzyme fractions by Reactive Red 120-agarose. We could not detect significant CANP I activity in any of the regions. CANP II activity was the highest in the pons-medulla and the cerebellum, with casein as substrate. In aging brain CANP II activity increased greatly in striatum, only slightly in cortex and pons-medulla. Enzyme property alterations are indicated by the substrate dependence of regional activity changes with age. The determination of regional inhibitor levels is in progress to clarify its role in the age-dependent alterations of CANP activity. (Supported by NIH AG05607).

517.8

LEUPEPTIN CAUSES AN ACCUMULATION OF PHOSPHORYLATED TAU AND UBIQUITIN IN RAT BRAIN. G.O. Ivy, K. Kitani and Y. Ihara. Life Sciences, Univ.of Toronto, Toronto, Ont. M1C 1A4 and Tokyo Metropolitan Inst. Gerontology, Tokyo-173, Japan.

In Alzheimer's disease (AD) and to some extent in aging, neurofibrillary tangles (NFT), often exhibiting the form of paired helical filaments (PHF) accumulate. Several laboratories have shown that the major antigenic components of PHF are phosphorylated tau proteins and that ubiquitin is present in NFT, possibly attached to phosphorylated tau itself. As an attempt at understanding the cellular mechanisms underlying NFT formation, we administered either the protease inhibitor, leupeptin or buffered saline intraventricularly to rats for two weeks using an osmotic mini pump. Brain tissue from these and normal aged rats was processed for immunocytochemistry using antibodies to PHF (affinity purified to react with phosphorylated tau) and ubiquitin. Many Purkinje cells in the cerebellum of leupeptin treated and aged, but not saline treated rats, displayed increased immunoreactivity to both antibodies. Anti-PHF antibodies labeled the perikarya and proximal dendrites while anti-ubiquitin stained perikarya, nuclei and portions of Purkinje cell dendrites. The finding that inhibition of thiol and some serine proteases causes a buildup of abnormal neuronal inclusions with antigenic similarities to NFT and PHF supports the protease inhibitor model of aging and AD (Ivy, 1987). Supported by NIA and NSERC.

517.9

LEUPEPTIN CAUSES SOME MANIFESTATIONS OF AGING IN THE RETINA
G. M. Smith and G. O. Ivy. Life Sciences, Univ.of Toronto, Toronto, Ont. M1C 1A4.

An accumulation of lipofuscin in the retinal pigment epithelium (RPE) is a hallmark of aging. This buildup may be due to a reduced ability of aged RPE cells to catabolize the contents of both phagosomes (mainly photoreceptor outer segment discs, PD) and autosomes. In this study, two month old Sprague Dawley rats were injected intraoccularly, every 24 hours for several days; one eye with lul of the protease inhibitor leupeptin (200 mg/ml) and the other with the same volume of buffered saline. Eyes from normal young and aged rats were also examined. RPE cells of eyes from aged and leupeptin treated rats contained more Periodic Acid Schiff positive granules than did RPE cells of saline treated eyes. Electron microscopic analysis confirmed the presence of numerous dense bodies in the RPE of leupeptin treated as compared to saline treated eyes; the majority of these appeared to be composed of PD at various stages of catabolism, as seen in untreated eyes from young rats. Other dense bodies displayed morphologies typical of lipofuscin seen in the aged rats. Further, varying degrees of photoreceptor degeneration were evident in many of the leupeptin treated and aged eyes. Together, the results indicate that lipofuscin accumulation in RPE cells may be largely due to a buildup of PD caused by decreased proteolytic activity and that photoreceptor degeneration can be caused by protease inhibition. Supported by NIA and NSERC.

517.10

LOSS AND RECOVERY OF STRIATAL DENDRITIC SPINES FOLLOWING LESIONS IN THE CEREBRAL CORTEX OF ADULT AND AGED MICE.
H-W. Cheng*, Y. Anavi*, H. Goshgarian, T.H. McNeill and J.A. Rafols. Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201; and Departments of Anatomy and Neurology, University Rochester School of Medicine, Rochester, NY 14642.

The dendritic spines of medium striatal neurons are postsynaptic targets to axon terminals of afferent fibers originating in the cerebral cortex, intralaminar thalamus, substantia nigra pars compacta and midbrain raphe. Lesions which involve some of these afferent fibers have been reported to result in substantial loses of striatal spines (Kemp, J.M., and T.P.S. Powell, Phil. Trans. R. Soc. Lond., B262:429, 1971). As part of our investigation in the aging striatum, we have examined by means of Golgi impregnations (Van der Loos modification) and computer-assisted morphometry (Bioguant IV), the effect of unilateral cortical lesions on the linear density of striatal spines in both adult (6 month) and aged (25 month) C57BL/6N mice. Three days after cortical lesions the density of spines per 20μm linear segment of dendrite is significantly ($p<0.005$) reduced in both the adult (by 30%) and aged (by 28%) groups compared to matched aged normals. The maximum spine loss (46%) is detected in both groups with survival of 10 days after lesion. A return to normal densities occurs in both groups between day 10 and 20 after lesions, and, at least, in the aged group complete recovery of the normal density is attained by day 35 postlesion. The present results reveal a capacity for plasticity in surviving striatal afferents as well as in the dendrites of striatal neurons in both adult and aged mice. In addition they also indicate a capacity of striatal spines in aged striatum to recover from cortical deafferentation with no apparent lag to initiate such a response as compared to the younger striatum. Supported by USPA Grants NS-14705, AG-00300-RCDA and AG-05445.

517.11

EFFECT OF AGE AND LONG-TERM OVARIECTOMY ON HYPOTHALAMIC NOREPINEPHRINE (NE) ACTIVITY ASSOCIATED WITH THE ESTRADIOL-STIMULATED LH SURGE.
K. Scarbrough and P. M. Wise, Dept. Physiology, School of Medicine, Univ. of Maryland, Baltimore, Md. 21201

Estradiol (E2)-induced LH surges are attenuated and delayed in middle-age compared to young rats. This age-related change in the pattern of LH release is associated with alterations in catecholamine turnover rates in hypothalamic nuclei known to be important in the regulation of the surge. Middle-aged long-term ovariectomized (OVX) rats (OVX at 3 mo.) exhibit E2-induced LH surges which are similar in timing and amplitude to those displayed by young rats. We tested the hypothesis that long-term OVX delays the age-related changes in the rate constants of NE activity in microdissected hypothalamic nuclei.

Young (3-4 mo.) and middle-aged (11-12 mo.) cycling rats were OVX. One week later these animals, plus a group of long-term OVX rats (11-12 mo.) were treated with capsules which produced plasma levels of E2 between 15 and 25 pg/ml. The rate constants of NE activity in the medial preoptic nucleus (MPN), the suprachiasmatic nucleus (SCN) and median eminence (ME) were determined at 09.00 and 15.00 h four days after E2 treatment using the α-methyl paratyrosine method. NE content was measured using HPLC. The right and left MPN and SCN were assayed separately.

In young rats, the rate constant of NE activity increased significantly from morning to afternoon in the ME and in the left but not the right SCN and MPN. Middle-aged short-term OVX-E2 treated rats failed to exhibit any diurnal rhythm in the rate constant of NE activity in the ME, SCN, or MPN. In contrast, middle-aged long-term OVX-E2 treated rats exhibited a diurnal pattern of NE activity in the ME similar to that of young animals but there was no diurnal rhythm observed in the SCN or MPN. Therefore, long-term OVX is associated with a sparing of NE function in the ME but not in the SCN or MPN.

Our data suggest that 1) long-term OVX prevents the age-related loss of the NE rhythm in the ME and 2) the maintenance of this ME rhythm may partially account for the ability of E2 to stimulate normal LH surges in these middle-aged rats. Supported by NIH AG02224, HD15955, HD07170.

517.12

BASAL GANGLIA/CEREBELLAR CALCIFICATION IN DOWN'S SYNDROME BRAINS OF THE YAKOVLEV COLLECTION.
C.D.West*, S.Colwell*, and V.Armbrustmacher* (SPON: E.Yeterian). Dept. Neurology (BIH), Harvard Med. Sch., Boston, MA 02115, Dept. Anatomy Boston Univ. Sch. Med., Boston, MA 01730, and the Armed Forces Institute of Pathology, Washington, D.C. 20306.

The distribution, severity and local neuronal effects of basal ganglia/cerebellar calcification/mineralization deposits were examined in 8 brains of the Yakovlev Collection from Down's Syndrome (DS) individuals, 2.5 to 56 years old. Four brains (2.5, 4, 16 and 29 years) had no deposits, or trace amounts. The others (6, 26, 47 and 56 years) had deposits increasing in severity with increasing age. The oldest had deposits in the cerebellum also.

The calcification/mineralization deposits were extraneuronal and often appeared as rows of spheroids organized along blood vessels. Heavier deposits appeared as disorganized, conglomerates of spheroids. The site of greatest concentration in DS brains was in globus pallidus. In controls trace deposits when found were located both in globus pallidus and/or caudate/putamen.

Planimetric measures of cell body and nucleus cross-sectional area, and measures of cell packing density revealed no differences among effected and non-effected DS brains or age-matched controls. Neurons were clearly absent only in the area of heaviest deposit (bilaterally) in the most severely involved brain, and only in portions of the neuropil which had an abnormal, gelatinous appearance.

517.13

DIFFERENTIAL EFFECTS OF AGING ON NEUROTRANSMITTER CELL LOSS OF THE SUBSTANTIA NIGRA AND STRIATUM IN MOUSE AND HUMAN BRAIN.
T.H. McNeill, L. Koek* and S.N. Haber. Depts. of Neurology and Neurobiology and Anatomy, Univ. Rochester, Rochester, NY 14642.

This study examined age-related cell loss of neurotransmitter neurons in the substantia nigra (SN) and striatum (ST) of mouse and human brain. Immunocytochemically stained neurons were counted in the SN and ST of C57 Bl/6 mice (3, 6, 10, 20, 25 and 30 mos.) and in the SN of human tissue (43-89 yrs.) obtained at an autopsy. Tissues were fixed in 4% paraformaldehyde and coronal sections through the rostral-caudal extent of the SN and ST were immunocytochemically stained for tyrosine hydroxylase, choline acetyltransferase, somatostatin and NPY. We found a significant loss of cholinergic neurons in the ST of aged mice (30 mos.) with no significant change in the number of somatostatin/NPY cells. In addition, while there was a small decrease (13%) in the number of dopaminergic neurons of the SN in aged mice (30 mo.) this decrease did not reach statistical significance. Similarly, in the human SN there was no significant change in the total number of dopaminergic neurons between 43 and 89 years of age. These data suggest that while there is a differential age-related cell loss in the ST of old mice, decreases in dopamine content of the ST previously described after adulthood in both rodents and man is not related to loss of dopaminergic neurons from the SN. Supported by PHS grants AG00300 and AG05445.

517.14

AGED RATS SHOW INCREASED BASAL LEVELS OF ADENOSINE 3',5'-MONOPHOSPHATE (cAMP) PRODUCTION WITHIN THE OLFACTORY BULB (O.B.).
**T. Mencio-Wszalek, D.E. Dluzen and V.D. Ramirez. **Institute of Animal Behavior, Rutgers, The State University of New Jersey, Newark, N.J.,07102. Department of Physiology, University of Illinois, Urbana, IL.,61801.

In the present experiment, O.B. were dissected out of young (less than 100 days of age) and aged (greater than 500 days of age) rats. Homogenized O.B. tissue extracts were analyzed by radioimmunoassay to determine the basal levels of cAMP production. The cAMP assay used an antibody that was developed and validated in our laboratory and had a sensitivity of 0.05 picomoles/tube at 92% binding. All values were corrected for the small amount of endogenous cAMP present in control samples by incubation without ATP. The basal levels of cAMP production were as follows: AGED RATS (n=5), Mean ± SEM = 37±2 picomoles cAMP produced/mg protein/minute; YOUNG RATS (n=4), Mean ± SEM = 21±4 picomoles cAMP produced/mg protein/minute. These values represent a statistically significant ($p<0.008$) increase, and indicate that in contrast to the majority of normal aging systems which exhibit deficits with age, the basal levels of cAMP production are actually increased in aged compared to young rats.

517.15

A BIOCHEMICAL MARKER OF NEURONAL AGING IN NORMAL HUMAN BRAINS. V.M. Ingram and B.J. Blanchard*. Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

An age-related additional phosphorylation of the 200kD neurofilament protein has been observed in normal human fixed brain tissue from individuals older than 60 years. We have studied in particular the axons of cerebellar basket cells. Only about half of the basket cell axons show this phenomenon. The probe is the Sternberger-Meyer Co.'s monoclonal antibody SMI-34, which also reacts with the neurofibrillary tangles of Alzheimer's disease, probably a phosphorylated tau protein, Our aged axons do not stain for tau. All human basket cell axons at all ages examined contain another phosphorylated NF200 epitope detectable by mAb SMI-31. Only the SMI-34 epitope appears in an age-related pattern and by a mechanism to be determined.

In sections of brainstemfrom Alzheimer'sdisease and from older Down's syndrome individuals certain axons in the medial longitudinal fasciculus and the mesencephalic trigeminal tract react strongly with SMI-34, as well as with SMI-31. In chicken and rat cerebella the SMI-31 and SMI-34 epitopes appear after hatching/birth and, in contrast with the human, appear at the same time. We are determining the structure and location on NF200 of the two epitopes and are purifying the kinases involved.

517.16

DEGENERATION IN CHOLINERGIC NUCLEI OF THE FOREBRAIN CORRELATE WITH COGNITIVE IMPAIRMENTS IN AGED RATS. W.Fischer*, F.H. Gage and A. Björklund (SPON: ENA). Dept. of Med. Cell. Res. Univ. of Lund, Sweden

Mice and rats display age-dependent declines in parameters of cholinergic function in various neocortical, hippocampal and striatal regions. Morphologically, neuronal atrophy and cell loss in the cholinergic neurons of the basal forebrain and in the striatum of aged rats and mice have previously been reported. In this study, degenerative changes in the forebrain cholinergic nuclei (medial septum, diagonal band of Broca, nucleus basalis and striatum) were analyzed morphometrically in behaviourally characterized aged rats. In all four regions the acetylcholinesterase-positive neurons were reduced in both size and number in the aged (24 months old) rats as compared to the young (3 months old) controls. The overall reduction in cell size amounted to between 20 and 30%, and the overall reduction in cell number to between 27 and 45%. Impairment in learning and memory performance in the aged rats, as assessed in the Morris'water-maze task, was significantly correlated with cholinergic cell size and cell number in the medial septum, the diagonal band of Broca and in the striatum. In nucleus basalis there was a trend in the same direction but did not reach significance. In contrast to these degenerative changes in the cell body regions, no significant differences in cortical or hippocampal choline acetyltransferase activity were detected biochemically between the young and aged rats, and the enzyme levels did not correlate with the degree of behavioural impairments in the aged rats.

The present results provide evidence that all major components of the forebrain cholinergic system undergo degenerative changes with age, and that these atrophic changes are correlated with the decline in spatial learning and memory in the aged rat.

517.17

MAO IN AGED FISHER 344 RATS. V. Luine and M. Hearns.* Hunter College, New York, N.Y. 10021.

Activity of Type A monoamine oxidase (MAO) was measured in brain areas of young and old (3.5 and 25 months) Fisher 344 female rats. Most areas examined are involved in regulation of the hypothalamic-pituitary-gonadal axis. Young rats were sacrificed in the diestrus, proestrus or estrus phase of the estrous cycle while all aged rats were in the recurrent pseudopregnant state. Sampling was by the punch out technique, and maximal in vitro MAO activity, using serotonin as substrate, was measured in the frontal cortex (FCtx), striatum (St), suprachiasmatic nucleus (SCN), bed nucleus of the stria terminalis (Nist), medial preoptic nucleus (mPOA), periventricular area of the POA (PVPOA), arcuate-median eminence area (Ar-ME) and the anterior, dorsomedial and ventromedial nuclei of the hypothalamus (AH, DMN and VMN). Comparisions between all aged and young showed changes in only 1 of the 10 areas: SCN activity was decreased by 30% in the aged females. Age related changes were evident in some areas related to neuroendocrine function when estrous state was considered: these included the mPOA, DMN, VMN and Ar-ME. Neither age nor age vs. estrous cycle variations were found in the Nist, AH or PVPOA. In the FCtx and St, areas outside of neuroendocrine regulatory centers, no differences in MAO were found. These results suggest that changes in MAO activity during aging are most evident in brain areas related to endocrine function. (Protocols in accordance with Federal and Society guidelines).

517.18

GRAFTS OF FETAL BASAL FOREBRAIN: SURVIVAL AND GROWTH IN THE ADULT AND AGED HIPPOCAMPUS. K.S Chen,* and F.H. Gage. (SPON: W.C. Wiederholt) Dept. of Neurosciences (M-024), UCSD, La Jolla, CA 92093.

Fetal basal forebrain neurons grafted to the adult hippocampus will exhibit markedly better survival and fiber outgrowth into the host hippocampus following a fimbria-fornix lesion (Gage and Bjorklund,1986). We find that fetal basal forebrain grafts to the hippocampus of aged rats also exhibit better survival following a fimbria-fornix lesion. In addition there is greater graft survival and outgrowth in the intact and denervated adult hippocampi than in the intact and denervated aged hippocampi respectively.

Adult and aged (20 month) Fisher 344 rats with unilateral aspirative lesions of the fimbria-fornix received 3ul cell-suspension grafts taken from the septal region of fetal rats (E14-E16). The cell suspensions were implanted bilaterally into the hippocampus. Following a two month survival period the average graft volume in the hippocampus, denervated at the time of grafting in the adult and aged brains, was significantly larger than the graft volume in the contralateral hippocampus. A lesion of the contralateral fimbria-fornix was made one week prior to sacrifice in order to assess the fiber outgrowth from the graft into the contralateral hippocampus without the presence of intrinsic cholinergic fibers. Immunohistochemical staining for choline acetyltransferase positive cells revealed the presence of graft-derived cholinergic cells in the denervated and non-denervated hippocampi of both adult and aged rats. Staining for acetylcholinesterase revealed substantial graft survival and fiber outgrowth from the graft into the lesioned as well as intact hippocampus of adult and aged brains. There was a significantly larger graft volume and fiber outgrowth in the adult brain. Therefore, there appears to be a loss of plasticity in the aged brain, perhaps as a result of decreased levels of trophic factors.

517.19

GLUTAMATE NEUROTOXICITY DURING DEVELOPMENT & AGING. C. Peterson, J. Neal*,[1] and C. Cotman Dept. Psychobiol., Univ. of CA at Irvine and [1]Dept. of Neurol. Surg., USC Sch. Med., Los Angeles.

Glutamate excitotoxicity has been implicated as an important factor in hypoxic-ischemic neuronal death. The resistance of young animals to hypoxia has been well described, but whether this is related to reduced glutamate receptor activation in the immature CNS is unknown. To clarify this issue, the binding of radiolabelled glutamate to the NMDA (N-methyl-D-aspartate) receptor was determined in the brains of the developing, mature and aged rodent.

Glutamate binding to the NMDA receptor increases from postnatal day 0 (14.7%), 7 (55.2%), 14 (79.0%) and 21 (93.7%) until it reaches adult levels at 90 days (100%) and then declines by 10 (-41%) and 30 months (-56%) of age. With advancing age the affinity of glutamate binding to the NMDA receptor increases despite a reduction in the number of binding sites. To determine whether development of glutamate binding correlates to NMDA neurotoxicity, an *in vitro* neuronal preparation was used.

Hippocampal neuronal cultures were prepared from rodent embryos (18-20 days). At certain days after plating, cultures were acutely treated with various concentrations of NMDA (500, 50, 0.5, 0.05 and 0.05 uM) for 5 min and alterations in cellular morphology and cell viability were noted 24 hrs later. Cultures which had been plated 5 to 9 days earlier displayed no alterations in morphology or cell survival. However, cells plated 12 days earlier showed marked dendritic degeneration and neuronal swelling while those plated 14 days earlier had widespread neuronal loss. Increasing NMDA concentrations leads to a decline in cell survival. This finding suggests that neurotoxicity due to NMDA receptor activation may be developmentally regulated.

Thus, the degeneration of hippocampal neurons in culture after acute NMDA treatment is age-dependent. This *in vitro* vulnerability correlates with the *in vivo* development of glutamate binding to NMDA receptors. The mechanisms of excitotoxicity in neuronal cultures may help develop potential strategies to prevent or reverse glutamate-induced neuronal degeneration.

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518.1

DEVELOPMENTAL REGULATION AND CHARACTERIZATION OF HIGH AFFINITY LAMININ RECEPTORS IN CHICK AND RAT CNS. S. Gee*, Philippe Douville* and S.Carbonetto (SPON: C.M. Pover). Neurosciences Unit, Montreal General Hosp. Res. Inst. and McGill University, Montreal, Canada.

Laminin (LN), an extracellular matrix (ECM) glycoprotein, promotes neural cell attachment, migration and neurite outgrowth. We have previously demonstrated 67 and 120 kDa high affinity LN receptors in neurons (Douville et al., 1987, Soc. Neurosci. Abs., 13: 1982).

In these experiments, detergent extracted membrane proteins were electrophoresed, electroblotted and probed with ^{125}I-LN. The most prominent band in autoradiographs is a 120 kDa protein that is detected in a variety of chick tissues, cultured neurons and astrocytes, and in several neural and nonneural cell lines. LN binding to this protein, which can be surface radioiodinated in optic lobe neuronal cultures, requires divalent cations and is inhibited by high concentrations of salt. This protein is apparently distinct from the chick integrin laminin receptor (Smalheiser and Schwartz, 1987, P.N.A.S., 84:6457-6461).

Preliminary experiments have shown that the apparent molecular weight of the 120 kDa receptor in chick retina decreases during development. Early in development the receptor migrates as a heterodisperse band(s), which later becomes more well defined. The regulation of these receptors may be important for the interaction of neural cells with LN during development.

518.2

A NEW CATALYTIC SITE ON Na^+,K^+-ATPase IN THE BRAIN OF GUINEA PIG FETUS: AFFINITY TO STROPHANTHIDIN. O.P. Mishra and M. Delivoria-Papadopoulos, Univ. of Pa. Sch. of Med., Dept. of Physiol., Phila., PA 19104

In order to understand the bioenergetic efficacy of the fetal brain Na^+,K^+-ATPase function, the present study examines and compares the affinity of cerebral Na^+,K^+-ATPase of fetal and adult guinea pig to its specific inhibitor, strophanthidin. The activity of Na^+,K^+-ATPase was determined in fetal and adult brain membrane preparations by measuring the rate of ATP hydrolysis in the presence of concentrations of ouabain and strophanthidin ranging from 10^{-10} M to 10^{-2} M. The ouabain or strophanthidin sensitive activity was referred to as Na^+,K^+-ATPase activity. There are three catalytic sites on the fetal brain enzyme compared to only two in the adult. $K_{0.5}$ values for strophanthidin inhibition in fetal brain were: 9.5×10^{-10} M, 6.5×10^{-7} M, and 5.5×10^{-5} M; in adult brain, the values were 6.5×10^{-7} M and 5.5×10^{-5} M. The first $K_{0.5}$ value in fetal brain is 1/1,000th to 1/100,000th of the adult values. It is proposed that a highly sensitive additional site of strophanthidin-binding, an additional phosphorylation site, and an additional site of ATP-binding are required for the early functional maturation of brain during the period of rapid brain growth in this precocial species, the guinea pig, and modified later in life. These additional mechanisms of Na^+,K^+-ATPase will be of added advantage to the fetal brain under conditions such as hypoxia/ischemia which lead to lower cellular concentrations of ATP. (NIH #R01 HD-20337 and HL-37421).

518.3

SPATIOTEMPORAL VARIATIONS IN STABLE AND ALKALI-LABILE GANGLIOSIDES FROM RETINA AND BRAIN OF CHICK EMBRYO. L.N. Irwin and C. Hanlon*. Dept. Biology, Simmons Col., Boston, MA 02115.

Interest in the possibility that gangliosides might play a role in the differentiation or synaptic specificity of the nervous system has prompted us to analyze the content and distribution of both stable and alkali-labile gangliosides in the developing retina and brain of the chick embryo. Retinas were removed from 12-day embryos and dissected into quadrants. Gangliosides were extracted, purified, and developed on HP-TLC plates in two dimensions (Irwin et al. 1985. J. Neurosci. Res. 13:591), then quantified by scanning with a Zeineh SLR-2D/1D Soft Laser Densitometer. Over 20 distinct gangliosides were present in all four quadrants, but quantitative differences among quadrants were evident. In particular, a ganglioside migrating in the position of GT3 was relatively more concentrated in the dorsal than in the ventral quadrant. Alkali-labile gangliosides were detected by exposing the plates to ammonia vapor between the first and second dimensions (Somnino et al. 1983. Analyt. Biochem. 128:104). At least seven spots apparently corresponding to alkali-labile gangliosides were revealed. Our results suggest that the nature and composition of the ganglioside population varies both temporally and topologically during critical stages of neurogenesis. (We thank Biomed Instruments, Inc., Fullerton, CA, for the loan of computer equipment and image-processing software for the quantitative analysis of two-dimensional ganglioside chromatograms).

518.4

ALTERATION OF 3-HYDROXYBUTYRATE INCORPORATION INTO LIPIDS OF THE DEVELOPING RAT BRAIN BY GLYCEROL. M.C. McKenna,* J.T. Tildon* and L.I. Bezold* (Spon: G. Bergey). Dept. of Pediatrics, Univ. Md. Sch. Med., Baltimore, MD 21201.

Several reports have demonstrated that the ketone bodies, acetoacetate and 3-hydroxybutyrate (3-HOB) play an important role in the metabolic homeostasis of the developing central nervous system. Earlier reports from our laboratory showed that increases in plasma glycerol can have profound anti-ketonemic effects (Roeder, L.M. et al., J. Nutr. 112:1273, 1982). In this study we measured the effect of intraperitoneal injection of glycerol on the rate of 3-hydroxy-3^{14}C butyrate incorporation into brain lipids in 11 and 18 day old rat brains in vivo. The percent radioactivity incorporated into individual lipids was also determined. Plasma levels of 3-HOB decreased from 2.24 to 0.56 and 1.38 to 0.41 mM in 11 and 18 day old animals, respectively. The rate of incorporation of 3-HOB into lipids was decreased about 5 fold at both ages. In 11 day old rats glycerol treatment significantly increased the amount of radioactivity incorporated into neutral lipids, sulfatides and phosphatidyl inositol and decreased the amount incorporated into phosphatidyl choline and phosphatidyl ethanolamine. In 18 day old rats only the incorporation into phosphatidyl inositol was altered. These results suggest that decreases in circulating levels of 3-HOB lead to significant alterations in the synthesis of brain lipids from 3-HOB and younger rats may be particularly vulnerable to these effects. (NICHD-16596)

518.5

DEVELOPMENTAL CHANGES IN HEART PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE (PAM) ACTIVITY AND mRNA LEVELS. L'H.Ouafik*, V.May and B.A.Eipper. Neuroscience Dept., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Peptidylglycine α-amidating monooxygenase (PAM; EC 1.14.17.3) catalyzes the α-amidation of bioactive peptides. The PAM precursor predicted from the sequence of a cDNA cloned from bovine neurointermediate pituitary is a 108 kDal protein containing a hydrophobic putative transmembrane domain near its C-terminus. High levels of membrane-associated PAM activity and PAM mRNA have been identified in adult rat heart atrium (Eipper et al. J. Biol. Chem. in press 1988). We have quantitated soluble and membrane-associated PAM activity and examined the levels and forms of PAM mRNA in the rat heart during development. In atrium, the highest levels of PAM mRNA were found at embryonic days 18-20 (E18-E20); the levels declined dramatically at birth and gradually increased to the high levels found in adult atrium by postnatal day 14 (P14). In ventricle, the highest levels of PAM mRNA were found at E14-E16; the levels diminished at birth, transiently increased until P7 and then declined to the low levels seen in adult ventricle by P14-P21. Both 3.6 and 3.8kb forms of PAM mRNA were observed in atrium and ventricle, although their ratio varied significantly during the days studied. For atrium and ventricle, a soluble form of PAM activity predominated at E14-E16. Membrane-associated PAM activity was the dominant form from E18 to adult.

518.6

EFFECT OF NERVE GROWTH FACTOR ON THE BIOSYNTHESIS ACTIVITY OF PTERINS IN PC12h PHEOCHROMOCYTOMA. N.Nakanishi, H. Suzuki*¶, K.Akiba-Aono*¶, K.Hirayama*, K.Ozawa*, and S. Yamada*. Depts. of Biochem., and ¶Pedodont., Meikai Univ. Sch. Dent., Sakado, Saitama 350-02, Japan.

PC12h pheochromocytoma is a subclonal line of PC12, and tyrosine hydroxylase of PC12h was known to be induced by nerve growth factor (NGF) treatment of the cells. We examined the NGF effect on tetrahydrobiopterin (BPH4), which is a specific cofactor (electron donor) for aromatic amino acid hydroxylases, and found that BPH4 level was transiently increased by NGF with the maximal level of about 150 ng/mg protein (2-fold over the control) at 24 h after the treatment was initiated. Then we examine the NGF effect on the activity of the cells for synthesizing pterins. Extracts from the cells were incubated with GTP, a substrate of GTP-cyclohydrolase which catalyzes the first step of BPH4 biosynthetic pathway, and the products were analyzed by high-performance liquid chromatography. NGF increased the activity of GTP-dependent synthesis of pterins: extracts from the NGF-treated cells (50 ng/ml, 24 h) showed about 2-fold greater activity than that from the control cells did. The results suggest that the NGF-induced increase in BPH4 is due to the increased activity of pterin biosynthesis.

518.7

THE DISTRIBUTION OF GAP-43 IN THE MONKEY BRAIN. K. Andruschak, L.I. Benowitz, K.L. Moya and S.N. Haber. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY and Dept. of Psychiatry, Harvard Med. Sch., Boston, MA.

GAP-43 in a neuron-specific phosphoprotein associated with the development, regeneration, and modulation of synaptic relationships. Using immunohistochemical techniques, the distribution of this protein was studied in the brains of adult rhesus monkeys.

Within the sub-cortical telenecephalon, GAP-43-like immuno-reactivity was present in the ventromedial striatum, the olfactory tubercle, the substantia innominota, the bed nucleus of the stria terminals, and portions of the amygdala. Particularly dense diencephalic staining was observed in midline structures such as the periventricular n. the dorsomedial n. of the thalamus, the paratenial n., and in midline hypothalamic nuclei. Midbrain and brainstem nuclei which showed dense staining included portions of the superior colliculus, the substantia nigra-pars reticulata, interpeduncular n. dorsal raphe, parabrachial n., n. of the solitary tract, and dorsal motor n. of the vagus. In addition portions of the neocortex and hippocampal formation showed dense staining.

The distribution of GAP-43 in the adult monkey brain to some extent parallels that reported in the adult rat (Benowitz, et al., J. Neurosci. 8, '88). Concentrations of the protein are high in areas regarded as having limbic or associative functions. However, unlike the rat several regions associated with motor and somatosensory functions also showed relatively dense GAP-43-like immunoreactivity. The more extensive pattern of GAP-43 staining in the primate compared to that observed in the rat may reflect an increase of integration or plasticity of these nuclei.

518.8

MK-801 BINDING SITES ARE PRESENT EARLY IN DEVELOPING RAT BRAIN. A.M. Morin, H. Hattori* and C.G. Wasterlain*. Neurology Res. Lab. and Epilepsy Res. Lab., VAMC, Sepulveda, CA 91343, Dept. of Neurology, UCLA Sch. of Medicine.

MK-801, an N-methyl-D-aspartate (NMDA) antagonist, protects against hypoxic-ischemic brain damage in neonates (Hattori, et al. abst. these meetings). ^{3}H-MK-801 (3nM) binding sites are present as early as 7 days of age in cortex (91.9±21.6 fmol/mg prot.n=5) and in hippocampus (154.0±8.5 fmol/mg prot.n=5). Assay of adult brain areas gave the following: cx, 254.5±15.7 fmol/mg prot.(n=5) and hp, 404.4±88.5(n=5). Comparison of brainstem of 7 day old neonate (63.3±3.0 fmol/mg prot.n=5) and adult (26.8±6.9 fmol/mg prot. n=5) indicated a significant decrease in binding ($p<0.05$) in adult. Glutamate or glycine (10uM) stimulated binding in washed (8x) hp membranes ($p<0.05$; glu, 132.7±16.5% of baseline binding and gly, 135± 15%). Binding in cx increased but did not reach significance (glut, 118.6±19.9%, gly, 123.2±10.5%) at this age but did so at 10 days of age. Phencyclidine (1uM) reduced ^{3}H-MK-801 binding in cx (9.9±10.5%) and hp (15.6±2%) while Mg ions (100uM) completely blocked binding. The data support the presence of NMDA coupled MK-801 binding sites early in brain development.

518.9

MOLECULAR FORMS OF TRANSIENT ACETYLCHOLINESTERASE IN THE DEVELOPING RAT BRAIN. C. Gorenstein, K.A. Gallardo* and R.T. Robertson. Depts. of Pharmacology and Anatomy and Neurobiology, University of California, Irvine. Irvine, CA 92717.

During development there is a characteristic transient appearance of acetylcholinesterase in the primary sensory regions of cerebral cortex and dorsal thalamus. It is not known, however, whether unique molecular forms of acetylcholinesterase are transiently expressed in these regions. Using sucrose gradient ultracentrifugation we have determined the molecular forms of acetylcholinesterase at various stages of brain development, in regions that express transient and continued enzyme activity.

Brain regions were homogenized in the presence and absence of 1% triton X-100 and the extracts analyzed on 5-20% sucrose gradients. In the mature brain, two major molecular forms of acetylcholinesterase were resolved from the soluble and detergent extracted tissue. The 10 S and 4 S forms predominated in the soluble extracts, while the 10 S form predominated in detergent extracted tissue.

Analysis of the transient acetylcholinesterase indicated that it also consisted of 10 S and 4 S forms, suggesting that the appearance of transient acetylcholinesterase does not represent the expression of novel forms of the enzyme.

Supported by grants from NIH (NS 25674) and NSF (87-08515).

518.10

DEVELOPMENT OF CHAT ACTIVITY AND CYTO-ARCHITECTURE IN THE FOREBRAIN OF THE SNELL DWARF MOUSE. C.F. Höhmann, G. Forloni, B. O'Hara*, L.E. Wilson*, M.L. Oster-Granite, J.D. Gearhart *and J.T. Coyle. Depts. of Physiology and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Dwarf (dw/dw) is an autosomal recessive mutation in Snell-Bragg mice that results in a nearly complete loss of acidophils from the anterior pituitary with accompanying hypothyroidism, dwarfism and reproductive failure. Adult dw/dw mice exhibit several CNS abnormalities, including decreased cholineacetyltransferase (ChAT) activity, increased somatostatin (SOM) and disrupted cytoarchitecture in some forebrain areas. In the present study, we examined the generation of these three abnormalities during ontogeny. We monitored ChAT activity, SOM immunoreactivity (SOM-IM) and acetylcholinesterase (AChE) histochemistry as well as Nissl staining in cortex, hippocampus and striatum of dw/dw and unaffected littermates at 7, 14, 30 and 60 days postnatal (PND). The specific activity of ChAT, in cortex or hippocampus, did not differ significantly between dw/dw and littermate controls. In contrast, beginning with PND 14, ChAT activity was significantly reduced in the striatum of dw/dw. AChE showed increased staining in layer IV of somato-sensory cortex in the adult dw/dw animals. The distribution and number of SOM-IM cells were not changed at any age in any forebrain region examined. However, a thinning of the supragranular layers of cortex, accompanied by poorly defined boundaries between layers, became apparent in Nissl stained sections by PND 14. We conclude that the dw/dw mouse does not exhibit the correlative relationship between ontogenetic cholinergic deficits and somatostatin and cytoarchitectonic abnormalities that we have previously observed in mice with neonatal basal forebrain lesions.

518.11

CORTICOSTERONE AND GROWTH HORMONE RESPONSES TO VARIOUS EXPERIMENTAL PROCEDURES IN NEONATAL RAT PUPS. S.M. Specht, C.L. Kirstein, C.M. Kuhn and L.P. Spear. Dept. of Psychology and Centers for Developmental Psychobiology and Behavioral Neuroscience, SUNY, Binghamton NY 13901 and Dept. of Pharmacology, Duke Univ., Durham NC 27710.

Intraoral cannulations require minimal time to administer and are typically assumed to be relatively unstressful in neonatal rat pups. To test the latter assumption, corticosterone (Cort) and growth hormone (GH) levels were measured in 4-day-old rat pups placed in an incubator for 15 or 60 min following: 1) no treatment; 2) subcutaneous (sc) injection of 0.9% NaCl; 3) anterior tongue cannulation; 4) posterior tongue cannulation; 5) ice anesthesia; or 6) ether anesthesia. Cort levels were elevated relative to non-treated controls 15 min after all treatments except sc injection. These levels remained elevated after 60 min in both cannulation groups and the ice anesthesia group. GH levels were significantly reduced at 15 min for all treatment groups except ice anesthesia. No significant differences in GH levels were observed at 60 min. These results indicate that intraoral cannulation as well as ice anesthesia might be moderately stressful procedures in neonatal rat pups. Whether ether anesthesia prior to cannulation will reduce these endocrine responses is currently under investigation.

518.12

5HT1A, 5HT1B and 5HT2 AGONISTS PRODUCE VARYING BEHAVIORAL RESPONSES IN 17-18 DAY OLD RAT PUPS. N.A. Frambes* C.L. Kirstein and L.P. Spear, Department of Psychology and Centers for Neurobehavioral Sciences and Developmental Psychobiology, SUNY-Binghamton, Binghamton NY 13901

There is an ontogenetic reversal in the influence of serotonergic (5HT) manipulations on mouthing and suckling during ontogeny, with these behaviors being facilitated by 5HT activation in neonatal rat pups, and inhibited in pre-weanling and weanling rats. In studies assessing the potential role of differential maturation of 5HT receptor subtypes in this ontogenetic reversal, deprived rat pups were tested both in the absence and presence of milk (low vs. high mouthing baselines, respectively) to assess both drug-induced increases and decreases in mouthing. At 3-4 days of age (P3-4), the 5HT1a agonist 8-OHDPAT inhibited mouthing, with the 5HT2 agonist DOI and the 5HT1b agonist mCPP conversely facilitating this behavior (Kirstein & Spear, in press). In contrast, at P17-18, no significant influence of DOI and mCPP were observed on mouthing, although mCPP did influence other behaviors (e.g., unusual posturing of the limbs). 8OHDPAT, however, still potently depressed mouthing at P17-18 while also resulting in poor control of the hindlimbs. Ontogenetic variations in the balance of activity among different 5HT receptor subtypes may contribute to the reversal seen in the influence of 5HT manipulations on mouthing and suckling during the neonatal to weanling age period.

518.13

EFFECTS OF PRENATAL TREATMENT WITH THE SEROTONIN AGONIST, 5-MT, ON APOMORPHINE-INDUCED HYPERACTIVITY. A. Shemer, E., Azmitia, Dept. Biology, and P. Whitaker-Azmitia, NY University.

We have developed an in vivo model for manipulating serotonin (5-HT) activity during development (Shemer et al., 1988). Pregnant rats are exposed to the 5-HT agonist 5-methoxytryptamine (5-MT) which causes the offspring to show reduced 5-HT outgrowth and deficits in behavior. The B_{max} of 5-HT receptors is also reduced as a result of the prenatal treatment. In order to test other possible changes in receptor characteristics, we challenged the drug treated neonates pharmacologically. The drugs used were acute 5-MT (1 mg/kg) to assess serotonergic sensitivity and apomorphine (1 mg/kg) to assess any changes in the dopamine system. Drug treated neonates were tested in a photoactometer at 30 days of age when there were no observable differences in activity between treated and control animals. The control animals were significantly affected by both drugs, 5-MT inhibited activity levels, and apomorphine increased activity levels. The drug neonates were unaffected by either drug. These results indicate that receptors in both the serotonergic and the dopaminergic systems were significantly less sensitive as a result of prenatal exposure to 5-MT.

Shemer, A., Whitaker-Azmitia, P.M. and Azmitia, E.C. (1988) Effects of prenatal 5-methoxytryptamine on serotonergic uptake and behavior in the neonatal rat. Pharmacol. Biochem. Behav., in press.

518.14

THE DEVELOPMENT OF STIMULATION-BOUND BEHAVIORAL ACTIVATION AND THE CORRELATED ANATOMICAL AND CHEMICAL CHANGES IN THE NUCLEUS ACCUMBENS. N.L. Goodless* and G.A. Barr (SPON: J. Gordon). Biopsychology Doctoral Program, Dept. Psychology, Hunter College, CUNY, N.Y., NY. 10021, USA and Dept. Psychiatry, Albert Einstein College of Medicine, Bronx, NY. 10461, USA.

In an attempt to identify the temporal and topographic patterns of behavioral activation, brief trains of electrical stimulation were administered to 3-, 5-, 7-, 10-, and 14-day old rat pups through stainless-steel bipolar electrodes directed at the nucleus accumbens (NAc). The resultant behavior was observed, first during a 2 min baseline period followed by three one-minute stimulation periods which alternated with one-minute non-stimulation periods. The occurrence of all behaviors was recorded every 10 sec. Pups 10 days of age and younger became behaviorally activated by stimulation and reliably emitted a series of behavioral responses including mouthing, licking, pawing, probing and locomotion. As the animal matured, electrical stimulation of the NAc elicited fewer activating behaviors. By the end of 2, weeks activation was no longer recognizable and subsequently disappeared. Grooming was the predominant behavior at this and did not appear to be concordant with stimulation. To relate the developmental changes of stimulation-induced behavioral activation with differential maturational patterns of the neuroanatomical and neurochemical systems within the NAc, the normal postnatal development of serotonin-, methionine enkephalin (Met Enk)-, and gamma-aminobutryic acid-like immunoreactivity was assessed. To relate the development of these neurotransmitters to morphological and metabolic characteristics of the NAc, we examined the ontogenetic patterns of Nissl stained cells, Bodian silver stained fibers and Cytochrome C oxidase activity. The findings revealed the differential ontogeny of various neurotransmitter systems in the NAc in the developing rat. Met Enk-like immunoreactivity was present at birth, while the other systems developed later, suggesting their possible participation in the loss of stimulation-induced behavioral activation.

518.15

RATIONALE FOR USING MATERNAL PLASMA GLUCOSE AND SCINTILATION COUNTS FOR DETERMINATION OF FETAL GLUCOSE UTILIZATION. D.R. Kostreva and J. Wood*. Dept. of Anesthesiology, Med. Col. Wis. and VA Med. Ctr. Milwaukee, WI 53295.

The operational equation of the Sokoloff [14C] deoxyglucose method (J. Neurochem. 28:897-916, 1977) is comprised of four components one of which, an integral, is derived from each animals plasma glucose and [14C] scintilation counts. Four pregnant rabbits were anesthetized, intubated, ventilated and their uterus was exposed. A connecting branch of the uterine vein was cannulated for sampling of venous blood from a single fetal-placental unit. The maternal femoral artery and vein were also cannulated for sampling. A bolus of [14C] deoxyglucose, 100 uCi/kg, was injected i.v. into the maternal circulation and the timed blood sampling procedure was initiated. The integrals were calculated for both the maternal arterial, and the fetal-placental plasma samples. The percent error in measuring glucose utilization using the maternal versus fetal plasma values was between 2 and 6% in three animals and 16% in the fourth. These differences were not statistically significant. Quantification of local fetal glucose utilization can therefore be made using the maternal plasma values.

518.16

FUNCTIONAL EFFECTS OF PRENATAL COCAINE EXPOSURE. T.A. Fico, L.A. Freed* and D.L. Dow-Edwards. Department of Developmental Psychobiology, NY State Psychiatric Instit., New York, NY 10032 and Laboratory of Cerebral Metabolism, State University of New York-Health Science Center at Brooklyn, Brooklyn, NY 11202.

Adverse neurobehavioral effects have been reported in infants prenatally exposed to cocaine. We have found that there are lasting changes in metabolic activity in several brain regions of the rat following exposure to cocaine during critical postnatal developmental periods (Dow-Edwards, et al , in press). We have now identified 2-deoxy-glucose changes in rat brain following prenatal cocaine exposure and correlated these changes with patterns of 3H-sulpiride binding.

Wistar rats were mated and housed under standard laboratory conditions. Dams received either 0 or 60 mg/kg po, from gestation day 8 through 22. Pair-feeding and surrogate fostering were used.

At 58-64 days of age, local rates of glucose utilization were determined in the male offspring using the deoxy-glucose method of Sokoloff et al. (1977). 3H-sulpiride binding was quantified and correlated with regions showing altered glucose metabolic activity. (Supported by NIDA grant #DA04118).

518.17

NIGRAL INFUSIONS OF MUSCIMOL IN RAT PUPS PRODUCE LOCAL CEREBRAL GLUCOSE UTILIZATION EFFECTS DIFFERENT FROM ADULT RATS. E.F. Sperber, L.L. Brown, J.N.D. Wurpel, S.L. Moshe, Albert Einstein College of Medicine, Bronx, NY 10461

Intranigral infusions of muscimol, a GABA agonist, are anticonvulsant in adult rats and proconvulsant in pups. This study localized the metabolic effect of nigral muscimol infusions in adult and 16 day old rat pup brains by using 14C deoxyglucose (DG) autoradiography. Unilateral cannulae were implanted in the substantia nigra (SN) of adult and rat pups. Adult rats had femoral catheters inserted for quantitative determination of local cerebral glucose utilization (LCGU) while pups were analyzed using a ratio to white matter. All rats were infused intranigrally with 100ng/0.25ul of muscimol (5 adults, 6 pups) or saline (5 adults, 4 pups) and injected with DG. Analysis of the adult autoradiograms showed changes in LCGU in globus pallidus, lateral habenula and deep layers of the superior colliculus (DLSC). In pups, changes in LCGU occurred in parietal cortex, dorsal striatum, globus pallidus, DLSC, posterior thalamic nucleus, forelimb sensory cortex and pontine reticular formation. The results suggest that the influence of nigral efferent pathways differ with age. The more widespread effects in pups may reflect the immaturity of inhibitory systems in the SN and its projection areas.

518.18

REGIONAL VARIATIONS IN TRITIUM QUENCHING IN RAT BRAIN DURING DEVELOPMENT. H.K. Happe and L.C. Murrin, Dept. of Pharmacology, Univ. Nebraska Med. Ctr., Omaha, NE 68105

Quantitative autoradiographic studies require correction for variations in quenching of tritium emissions by different brain regions. We investigated changes in regional tritium quenching during the first three weeks of neonatal life. Conditions were established for labelling the brain with [^{3}H]2-deoxyglucose (2DG) in neonatal rats using i.p. injections. Quench correction was determined by measuring 2DG labelled brain tissue before and after chloroform extraction (Brain Res. 336: 334, 1985). Image analysis used DUMAS from Drexel Univ.

Percent Tritium Quench by Region

	5 day	21 day	Adult
PC	28.1	35.4	40.0
BLA	26.5	31.3	36.1
CPu	27.3	40.3	33.7
IC	31.2	96.9	139.2
LH	33.9	42.1	72.8
CC	45.8	60.0	105.9

(PC = parietal cortex; BLA = basolateral amygdaloid n.; CPu = caudate-putamen; IC = internal capsule; LH = lateral hypothalamic area; CC = corpus callosum).

These studies demonstrate there is greater tritium quenching in white matter than in grey matter and that the level of quenching changes in particular brain regions during development, as would be expected with the development of myelination. Supported by NS23975.

519.1

THE SUPRACHIASMATIC NUCLEI (SCN) ARE ESSENTIAL FOR DAILY TORPOR AND CIRCADIAN BODY TEMPERATURE RHYTHMS IN SIBERIAN HAMSTERS. N.F. Ruby*, N. Ibuka*, B.M. Barnes*, J. Dark, and I. Zucker. Dept. of Psychology. Univ. of California, Berkeley, CA 94720.

Daily torpor was studied in hamsters, Phodopus sungorus, maintained in a short day photoperiod (8 hr Light/day) from weaning. Thirty animals implanted abdominally with radiofrequency transmitters were transferred to a cold chamber (15°C) at 60 days of age. Body temperature (T_b) and locomotor activity data were collected at 10 minute intervals using a telemetry system and stored by computer for subsequent statistical analysis. Animals manifesting two torpor bouts (T_b<30°C) in one week for two consecutive weeks received lesions of the SCN, pinealectomies, or sham operations. In the 15 weeks following surgery none of the animals with bilateral SCN lesions showed torpor, circadian body temperature, or activity rhythms as determined by Fourier analysis. All pinealectomized hamsters continued to show torpor for at least 6 weeks postsurgically, as did the sham-operated groups.

Daily torpor, once initiated, persists in the absence of the pineal gland and melatonin. Expression of torpor and manifestation of circadian body temperature and activity rhythms require intact suprachiasmatic nuclei.

Supported by NICHD Grant HD-02982.

519.2

PHOTIC AND NEURAL REGULATION OF PUBERTY ONSET IN MALE FERRETS. L.M. Sheppard and C.L. Sisk. Neuroscience Program and Dept. of Psychology, Michigan State University, East Lansing, MI 48824.

Transfer of male ferrets from short days (LD 8:16) to long days (LD 18:6) at 12 wk of age induces testis growth 4-6 wk earlier than in ferrets remaining in LD 8:16. Anterior hypothalamic lesions also induce precocious gonadal maturation in male ferrets. We compared within a single experiment the effects of photoperiod and hypothalamic lesions on puberty onset. Electrolytic lesions were aimed at the anterior hypothalamus of 13-wk-old ferrets housed in LD 8:16 to determine if their rate of maturity would be similar to that of other ferrets transferred from short to long days at 13 wk of age. Such a result would suggest that hypothalamic lesions mimic a photoperiodic response. Mean age at onset of testis growth was about 14 wk in ferrets transferred from short to long days, about 16 wk in ferrets in LD 8:16 with hypothalamic lesions, and about 18 wk in control animals in LD 8:16. There was considerable individual variation in onset and rate of testis growth among ferrets with lesions. Conclusions await histological evaluation of lesion placement. It will be important to determine if age at onset of testis growth correlates with lesion placement, particularly since the anterior hypothalamus contains structures associated with reproductive responses to photoperiod (e.g., suprachiasmatic nucleus).

519.3

DAYLENGTH DETERMINES THE INVOLVEMENT OF OPIOIDS IN STEROID-DEPENDENT CONTROL OF LH IN SYRIAN HAMSTERS. T.S.Juss*, M.H.Hastings* and J.Herbert*. (SPON: J.Hutchison). Dept. of Anatomy, University of Cambridge CB2 3DY U.K.

The role of endogenous opioids in the steroid-dependent control of LH was investigated by comparing the effects of castration, morphine and naloxone on LH secretion in photostimulated (PS, 16L: 8D), photoinhibited (PI, SD 8L: 16D) and photorefractory (PR, SD 8L: 16D) hamsters. Blood LH content was determined 4 days after castration or 30 min after drug administration.

In PS animals castration or naloxone adminstration increased, and morphine suppressed, serum LH. These manipulations were ineffective in PI animals maintained in SD for 12-18 weeks. Castration and drug responses were re-instated after 22 weeks in SD with the onset of PR (as determined by testicular recrudescence). The castration response therefore varied in parallel with that of opiatergic manipulations.

Exp. 2. PS or PI hamsters (after 8 weeks SD) were castrated and received sub-cutaneous testosterone implants. In PS and PR (22 weeks in SD) animals, LH secretion was responsive to opiatergic manipulations. In PI (12-18 weeks in SD) animals opiatergic manipulations were ineffective. However, the response to steriod withdrawal was present in both the PS and PR and also PI animals. Under inhibitory photoperiods steroidal inhibition of LH may therefore be independent of opioid function.

519.4

FAT PAD SPECIFIC CHANGES IN MASS, LIPOPROTEIN LIPASE ACTIVITY AND IN VIVO LIPOGENESIS IN SHORT PHOTOPERIOD-EXPOSED SIBERIAN HAMSTERS. T. J. Bartness, J. M. Hamilton, B. D. Goldman and G. N. Wade. Worcester Fnd. Exptl. Biol., Shrewsbury, MA 01545 and Univ. Mass., Amherst, MA 01003.

Short day-exposed Siberian hamsters initially decrease their body weight, a response reflected exclusively as a decrease in carcass lipid. However, with continued short day exposure body weight and carcass lipid return to long day levels. To examine whether the short day-induced changes in carcass lipid are uniformly distributed in all fat pads, adult male Siberian hamsters were housed in long (LD 16:8) or short (LD 10:14) days for 6, 12, 24 or 30 wk. After 6 or 12 wk short day exposure, body weight and carcass lipid were decreased. Fat pad mass, lipoprotein lipase (LPL) activity and *in vivo* lipogenesis were also decreased; however, the decreases were proportionally greater in the peritoneal fat pads (PFP; epididymal and retroperitoneal) than in the subcutaneous fat pads (SFP, dorsal and inguinal) relative to the long day controls. After 30 wk short day exposure body weight and carcass lipid were increased; however, the increases in mass and LPL activity of the PFP were proportionally greater than in the SFP relative to the long day controls.

519.5

EFFECT OF LGN LESIONS ON PHOTOPERIODISM AND BODY WEIGHT IN GOLDEN HAMSTERS. L.Smale, R.Y.Moore, and L.P.Morin. Psychiatry and Neurology Departments, SUNY, Stony Brook, N.Y. 11794-8101

Photoperiodic responsiveness in golden hamsters is mediated by a circadian timekeeping system that includes the suprachiasmatic nuclei (SCN). Input from the lateral geniculate nucleus (LGN) to the SCN influences entrainment, particularly in short daylengths (LD 8:16, Johnson,Moore and Morin, unpublished observations), and may therefore be an important element of the mechanism underlying photoperiodic time measurement. This hypothesis was tested by examining the effect of LGN lesions on photoperiodic responsiveness in male golden hamsters. Hamsters were given infusions of the neurotoxin n-methyl aspartate into the region of the LGN or sham lesioned while kept on a long daylength (LD 14:10). Two weeks later (wk 0), half of the animals from each group were transferred to short days (LD 8:16). Body weights and transscrotal testes length and width were measured every 3 weeks from wk 0 to wk 12, at which point running wheels were provided and locomotor rhythms recorded. Testes size did not change in hamsters kept in LD 14:10 but decreased substantially in groups kept in LD 8:16. However, regression was blocked in 2 and delayed in 2 of the 10 LGN lesioned hamsters in short days. After 12 wks in LD 8:16, LGN lesioned hamsters weighed substantially more than sham operated controls; in LD 14:10 body weights did not differ between theese groups.

519.6

PHOTOPERIODIC REGULATION OF STEROID RECEPTORS, SEXUAL BEHAVIOR, AND PITUITARY mRNA IN FEMALE GOLDEN HAMSTERS. E.L. Bittman, K. Hegarty*, M.Q. Layden*, and J.A. Jonassen*. Departments of Zoology and Physiology, University of Massachusetts, Amherst 01003 and University of Massachusetts Medical Center, Worcester 01655.

To examine neuroendocrine events which may underlie influences of daylength on behavior and synthesis of LH and prolactin (PRL), hamsters were ovariectomized and either left in long days (LD, 14L:10D) or transferred to short days (SD, 10L:14D). After 58 days, 5mm Silastic capsules containing 0%, 1%, or 5% estradiol (E2) were implanted at 1400h. Hamsters were injected with 0.5 mg progesterone at 44h and tested for receptivity at 48h. Capsules were removed and reimplanted 2 weeks later. After 44h, hypothalamus+preoptic area was dissected at 1400h for assay of nuclear estrogen (nEr, fmole/ug DNA) and cytosolic progestin receptors (cPr, fmole/mg protein). RNA was extracted from anterior pituitaries, dotted onto nitrocellulose, and probed sequentially with 32P-DNAs for rat LH-beta subunit, rat PRL, and oligo-dT. Results (mean±SEM) are listed below:

Group (%E2)	% in heat	nEr	cPr	relative LH mRNA	relative PRL mRNA
0% LD	-	5.0±1.5	4.6±.5	6.8±.6	12.0±3.1
SD	-	3.3±0.7	4.3±.3	1.0±.7	1.0±0.2
1% LD	57%	27.7±2.7	4.9±.7	2.9±.5	22.2±2.2
SD	22%	30.6±1.6	5.3±.5	1.8±.5	17.8±3.2
5% LD	100%	29.9±0.8	7.4±.8	3.8±.7	27.8±9.6
SD	50%	33.3±1.0	7.1±.6	0.4±.2	16.9±3.7

Short days decrease behavioral responsiveness to E2 without grossly influencing HPOA nEr occupancy or cPr induction, and reduce pituitary LH and PRL mRNA abundance through both E2-dependent and -independent actions. Supported by NIH HD20018 and HD20910.

519.7

SHORT DAYLENGTHS ALTER ELECTROPHYSIOLOGICAL RESPONSES OF HAMSTER SUPRACHIASMATIC NUCLEUS (SCN) NEURONS TO MELATONIN. B. Rusak and R. Mason*, Dept. of Psychology, Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4J1.

Several studies have suggested that the SCN is one target for melatonin effects that regulate seasonal reproductive cycles. We investigated whether melatonin alters firing rates of cells in the SCN of Syrian hamsters. Male hamsters were kept in a short photoperiod (10 h light daily for more than 150 days) to induce gonadal regression and spontaneous recrudescence. The reproductive systems of these hamsters become insensitive to exogenous melatonin. We compared the responses of their SCN neurons to those of animals housed in long days (14 h light), who are sensitive to melatonin. Extracellular recordings were made from hypothalamic slices in vitro to assess the effects of pressure ejection of melatonin (2 mM) on spontaneous firing rates. In long-day hamsters, melatonin evoked dose-dependent responses in approximately half the cells tested; unresponsive cells were encountered most frequently during the projected dark phase. About twice as many cells were suppressed as activated by melatonin. In contrast, nearly all SCN cells recorded in short-day hamsters were unresponsive to melatonin. These data suggest that functional insensitivity to melatonin may reflect in part a loss of neural responses to melatonin in the SCN.

Supported by NSERC and MRC of Canada, the Royal Society (UK), and Dalhousie RDFS.

519.8

THE PINEAL GLAND REGULATES REENTRAINMENT AND MELATONIN FACILITATES REST IN RATS. K.A.Dawson. Department of Psychology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

In an effort to elucidate the role of the pineal gland in the regulation of activity rhythms, 7 male Long Evans rats were subjected to pinealectomy by excision and 7 were sham controls. All animals had free access to running wheels in a colony room on LD 12:12 for 1 week and then the photocycle was reversed for 10 days. Daily melatonin injections were begun following return to the LD cycle and the rats were then subjected to a second reversal of the photocycle. Only within 5 days following reversal did pinealectomy significantly increase the proportion of active time in the light phase relative to active time in the dark phase. Melatonin significantly decreased active time in the light phase both before and after reversal. These results support the interpretations that exogenous melatonin facilitates rest during the light phase and the pineal gland regulates adjustment of activity rhythms to abrupt shifts in the environmental photocycle.

519.9

CARBACHOL MIMICS THE PHASE-SHIFTING EFFECTS OF LIGHT ON THE CIRCADIAN SYSTEM OF DJUNGARIAN HAMSTERS. B. E. F. Wee and F. W. Turek. Dept. Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

The cholinergic agonist, carbachol, mimicks the phase-shifting effects of light on (1) pineal NAT activity in rats and (2) the circadian rhythm of locomotor activity (CRLA) in mice and golden hamsters. These results suggest that the effects of light on the circadian system may be mediated by cholinergic mechanisms. The purpose of the present study was to determine whether the phase-shifting effects of light on the CRLA in Djungarian hamsters (Phodopus sungorus) can be mimicked by carbachol. The Djungarian hamster is unusual, because, unlike other species in which the phase advance region is limited to the late subjective night (SN), the phase response curve (PRC) to light in Djungarian hamsters has an additional small phase advance region in the late subjective day (SD).

Blind adult male Djungarian hamsters received intracerebroventricular injections (1 ul) of either 10 nmol carbachol or vehicle at one of 12 circadian times, and PRCs were generated. Carbachol induced significant phase delays at CT 12, 14, and 16 ($p < .01$) and small, but significant phase advances at CT 8 and 10 ($p < .05$).

The PRCs to light and to carbachol for the CRLA in the hamster both have phase delay regions in the early SN and small phase advance regions in the late SD. Although the carbachol PRC does not appear to have a phase advance region in the late SN as does light, these results indicate that carbachol mimics some of the effects of light on the circadian system in Djungarian hamsters and support the hypothesis that the phase-shifting effects of light on the circadian clock of mammals are mediated, at least in part, by cholinergic mechanisms.

519.10

ESTROGENS MODULATE CIRCADIAN LOCOMOTOR ACTIVITY IN THE SHORT PERIOD (Tau) MUTANT HAMSTER. E. J. Ampleford* and M. Menaker. (SPON.Hossein Hashemzadeh) Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Heterozygous tau mutant (T_s) golden hamsters display free-running periods of approximately 22h. Females entrained to a Light:Dark cycle display an exaggerated scalloped pattern of locomotor onsets with early onsets on days when estrogen levels are high (diestrus 2, proestrus) and later onsets when estrogen levels are low (estrus, diestrus 1). In T_s females, early onsets occur approximately 6h prior to lights off while late onsets occur up to 4h later. When ovariectomized, T_s females show a gradual reduction in overall activity and a progressive delay in the time of locomotor onset until reaching that normally associated with low estrogen levels. Implantation of silastic capsules containing estradiol increases the overall level of activity and restores the phase of the onset of locomotor activity to that associated with elevated levels of estrogen. In addition, ovariectomized estradiol treated females display large (>1h) changes in free-running period.

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519.11

CIRCADIAN MODULATION OF A HOMEOSTATIC DRINKING RESPONSE IN RATS. R.F. Johnson and A.K. Johnson. Depts. of Psychology, Pharmacology and The Cardiovascular Center, University of Iowa, Iowa City, IA 52242

We tested whether the amount of water intake to hypertonic saline injections is dependent on the circadian phase of the homeostatic challenge.

Baseline drinking of rats in an LD 12:12 cycle (onset 0600 hr) was monitored with lickometers to determine a peak (1800) and trough (0900) of drinking. A dose response of drinking to concentrations of hypertonic saline (1 ml/100 g bw of 0.9, 2, 4, 6, & 8% s.c.) in a 3 hr drinking test was obtained for both times. The rats drink more at 1800 than at 0900 hr ($p<.001$). The difference increased with increased concentrations of saline ($p<.01$). Low concentrations produced differences within the 1st hour postinjection and higher concentrations in the 2nd and 3rd hours. The differences were maintained even when the 1800 hr test was done under lights; rats drank more in the 2nd and 3rd hours of tests at 1800 hr than at 0900 hr, and the difference increased with concentration (0.9, 4, & 8%; $p<.01$).

The results suggest an interaction of circadian and homeostatic controls of drinking. Possible mechanisms of such an interaction will be discussed.

Supported by NIH HL14388.

519.12

RESPONSE OF DAILY BODY TEMPERATURE RHYTHMS OF AGED, YOUNG AND SCN-LESIONED RATS TO RESTRICTED FEEDING SCHEDULES. B.Tate-Ostroff and E.C. Walcott* Harvard Medical School, Boston, MA 02115; Mailman Res. Ctr., McLean Hosp., Belmont, MA 02178; Harvard College, Cambridge, MA 02138.

Age-related dysrhythmia may be the result of abnormalities in the SCN-based, light entrainable oscillator. Entrainment to food restriction schedules (FR) appears to occur through an oscillator anatomically distinct from the SCN. The objective of the present study was to examine the response of the body temperature rhythms of intact, SCN-lesioned and aged rats to FR. Rats exposed to LD 12:12 (lights on 0700) were placed on FR for 14 days, with food available from 1300-1500. Following FR the animals were given food ad libitum for 3 days, and then food deprived for 2 days. Body temperature was measured by telemetry. During both FR and food deprivation, animals from all groups showed elevations in body temperature during the light phase and preceding food availability by 2-4 hours, a pattern resembling that of activity bouts displayed by rats exposed to FR. The food-entrainable oscillator appears to be intact in the aged, dysrhythmic rats. Support by NSF grant DCB-85-08187 and AFAR grant to BTO.

519.13

CORRELATION OF SERUM CATECHOLAMINES WITH BEHAVIOR AS A FUNCTION OF GENETIC FACTORS AND TIME OF DAY. D. F. Peeler and I. Marawi* Univ. Miss. Med. Ctr., Jackson, MS 39216.

Serum epinephrine (E) and norepinephrine (NE) levels of male mice (n=126) from 9 inbred strains (C57BL/6, BALB/c and their 7 recombinant inbred strains) were determined for several times of day using HPLC with electrochemical detection. E and NE for each strain and time of day were compared with performance of these strains in previous studies of activity, emotion and learning.

NE typically was much higher than E in morning serum samples. Afternoon serum typically had higher levels of NE and lower levels of E than did morning samples. Strain E and NE levels were significantly correlated for morning but not for afternoon samples. Levels of neither hormone were significantly correlated between morning and afternoon.

Correlation was significant between the morning E level of each strain and one measure of emotion - tail rattling, but not with another emotion measure - defecation, nor with activity. Morning NE correlated with investigatory activity at the same time of day or late afternoon, but not at other times of day nor with locomotor activity or either measure of emotion. Morning levels of NE and E correlated significantly with later phases of shock avoidance learning. Afternoon NE levels correlated only with defecation scores obtained at the same time of day. Afternoon E levels correlated only with late afternoon locomotor activity.

Serum E and NE levels, and their relation to behavior, reflect an interaction of genetic factors and time of day.

519.14

RUNNING AND A MONOAMINE OXIDASE INHIBITOR ALTER SEROTONIN CIRCADIAN RHYTHMS IN HAMSTER SUPRACHIASMATIC NUCLEI IN DIFFERENT WAYS J.S. Kruse Center for Brain Research & Psychology Department University of Rochester, Rochester, N.Y. 14642.

Serotonin (5HT) fibers in the suprachiasmatic nuclei (SCN) mediate delays in free-running and entrained running wheel onset caused by monoamine oxidase inhibitors (MAOI). Increasing the running duration prior to MAOI treatment mitigates the delays. The present study examined the effect of 2 weeks of MAOI (10 mg/kg/day LY51641) and wheel running (DRUG), or 2 weeks of running alone (RUN), on the daily pattern of indoleamine concentrations within the SCN of Golden hamsters. Hamsters caged without wheels (CAGE) served as controls. SCN were microdissected at 8 equidistant times during the 14:10 L:D cycle. For each group at each time, n = 4. Samples were analyzed by high performance liquid chromatography.

DRUG, as compared to CAGE or RUN hamsters, had increased 5HT and decreased 5HIAA at all times of day. RUN hamsters had higher 5HT and 5HIAA than did CAGE hamsters during the dark when running occurred. These findings support the hypothesis that the 5HT projection to the SCN provides feedback information about locomotion to a circadian oscillator. Antidepressants may alter rhythms by disrupting a normal modulatory action of 5HT.

519.15

EFFECTS OF CHRONIC CLORGYLINE ON CIRCADIAN WHEEL RUNNING ACTIVITY IN NORMAL AND THYOPARATHYROIDECTOMIZED (TPX) RATS. **J. Schull, W. Duncan, E. Buhl*, D. Haverstick*, and J. Walker*. Haverford College, Haverford, PA 19041.**

Manic-depressive illness has been associated with thyroid abnormalities, shortened circadian periods of physiology and behavior, sex-specific abnormalities of motivation and activity, and heightened sensitivity to photo-stimulation. Abnormal reactions to anti-depressant medications have also been reported in manic-depressives, in whom mania and rapid-cycling may be triggered. Our previous findings suggest a causal role for the thyroid in normal and pathological control of circadian rhythms and behavior: TPX rats manifest shortened circadian periods of wheel running, sex-specific abnormalities of motor activity (elevated in males but not in females) and increased sensitivity to activity-suppressing effects of light. We now report that TPX males also display abnormal reactions to an antidepressant drug, the monoamine oxidase inhibitor clorgyline.

Forty-eight male Sprague-Dawley rats, half TPX and half Sham-operated, were housed in computer-monitored running wheel cages. The experiment was conducted in three stages. After two weeks of Entrainment (LD 12:12), animals were implanted s.c. with Alzet minipumps (Model 2002) delivering clorgyline (2 mg/kg/day) or saline. Following three weeks of Entrainment with Drug, the minipumps were removed and replaced, and animals were placed in Free Running conditions (constant dim red illumination) for 3 weeks.

TPX rats had increased activity levels ($p<.01$) and shortened circadian periods ($p<.005$) relative to shams, as previously observed. Activity levels increased for all groups over the two stages of Entrainment ($p<.01$). These increases were greater for animals receiving clorgyline ($p<.01$). After the transition to Free Running conditions, activity levels decreased in Sham-Saline, Sham-Clorgyline, and TPX-Saline animals ($p<.05$, N=12 for each group). However, activity increased further in TPXs receiving Clorgyline ($p<.05$, N=12). Clorgyline did not affect circadian activity periods in Sham or TPX animals during Free Run. Results suggest that thyroid abnormalities can influence reactions to clorgyline, and that clorgyline can elevate activity levels without affecting circadian periods.

519.16

EFFECTS OF p-CHLOROPHENYLALANINE ON CIRCADIAN RHYTHMS OF BODY TEMPERATURE, DRINKING AND ACTIVITY. J.M. Tomkowiak*, S. Kent and E. Satinoff. Dept. of Psychology, Univ. of Illinois, Champaign, IL 61820.

Serotonin (5-HT) has been implicated in the control of thermoregulation. para-Chlorophenylalanine (pCPA) is a tryptophan antagonist that depletes brain levels of 5-HT. We examined the effects of pCPA on the circadian rhythms of body temperature (Tb), drinking and activity of 9 unrestrained and unhandled male and female Long-Evans rats (b.w. 275-400 g). Tb was monitored by telemetry every 10 minutes, drinking and activity every 20 minutes for 12 days after a single pCPA injection (300 mg/kg i.p.). Rats were kept on a 12:12 LD cycle at an ambient temperature of 23±1°C with food and water available ad lib.

Compared to the saline day, pCPA increases the amplitude by .7±.1°C (± SEM) on day 1 ($p\leq.01$) and decreases the amplitude for the next 4 days ($p\leq.01$). Hour by hour analysis showed the increase in amplitude on day 1 is due to a decrease in Tb minimum and the decreases in amplitude on days 2 through 5 are due to an increase in Tb minimum and a decrease in Tb maximum. No significant effects were observed after day 5.

pCPA decreases the nocturnality of drinking from 83±2% to 63±7% on day 2 ($p\leq.05$), and to 63±3% on day 3 ($p\leq.01$). Nocturnality of activity decreased from 75±2% to 45-55% for the next 3 days ($p\leq.05$). No significant effects were observed after day 3.

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519.17

ENTRAINMENT BY FOOD OF CIRCADIAN LOCOMOTOR ACTIVITY RHYTHM IN THE CRAYFISH. F. Fernández de Miguel* and H. Aréchiga. Depto. Fisiología, Biofísica y Neurociencias, CINVESTAV, Apdo. Postal 14-740, México, D.F. 07000. México.

Although various sensory inputs may entrain circadian rhythms only light-dark cycles have been tested in crustaceans. In order to explore other entraining influences, the effect of food was studied on the locomotory rhythm of the crayfish Procambarus clarki. Number of movements was determined in actographic chambers in adults of either sex. Three experimental conditions were tested: a) continuous illumination (L:L), b) continuous darkness (D:D) and c) cycles 12:12 hrs L:D. A circadian period (τ) of 22.7±0.09 was determined in D:D:, in contrast to a τ = 24.8±0.02 hrs in L:L, which is in accordance with Aschoff's rule. Food was applied at different times under the three experimental conditions. Within the following hours a burst of locomotor activity ensued which lasted for 1.17±0.3 hours. It reappeared in phase during the following days. Entrainment by a single food pellet could last up to more than two weeks. The newly entrained rhythm displays peculiar circadian features: τ is longer in D:D (25.21± 0.36) than in L:L (23.26±0.17). In L:D τ is a function of the phase of the circadian cycle in which food is applied. Entrainment can be attained with food homogenates suggesting a chemical sensory input. These results suggest the interaction of two circadian systems in the control of crayfish locomotor rhythmicity.

519.18

CIRCADIAN VARIATION IN PLASMA MHPG LEVELS. **J.E.Piletz, A.Halaris, M.Marler*, J.F.Nash, and H.Y.Meltzer. Dept. of Psychiatry, Case Western Reserve Univ. and Cleveland Metropolitan General Hospital, Cleveland, Ohio 44109.**

Previous studies have demonstrated a circadian rhythm in the plasma norepinephrine metabolite, MHPG, from normal adults. Relative to healthy controls (n=12), depressed patients (n=43) displayed dysregulated rhythms in total MHPG. Patients circadian values were only weakly fit to a sinusoidal model (22/43 patients yielded $p\leq0.1$ for a sinusoidal fit as compared to 10/12 controls). Patients (n=23) completing a 6-week treatment course with desipramine did not show a significantly improved sinusoidal fit. The circadian aberration in patients was difficult to quantitate because only one 24 hr period was studied. To further study this, 48 hr blood samples were collected and free, sulfo-conjugated and glucuronide-conjugated MHPG fractions were differentiated. Previous reports indicated that plasma levels of sulfo-MHPG and free MHPG are of CNS origin, while gluco-MHPG is believed to be largely of peripheral origin. We also assessed two HPA measures (plasma cortisol and prolactin) which normally show an opposite circadian phase as MHPG. Preliminary results suggest that there is a circadian pattern in free and sulfo-conjugated but not glucuronide-conjugated MHPG. The results help to clarify the nature of the apparent dysregulated MHPG rhythm in depressed patients.

519.19

CIRCADIAN EFFECTS ON FATIGUE AND SUSTAINED PERFORMANCE (SP). W.N. Tapp and B.H. Natelson. Primate Neurobehav. Unit, VA Med. Ctr. and Dept. Neurosciences, New Jersey Med. Sch., East Orange, NJ 07018.

We have developed a monkey model for studying circadian effects on performance. However, in many of the natural uses for this information -- jet-lag, shift work, and SP -- fatigue is also an important influence on performance. Therefore, we conducted an experiment to explore the effects of SP on fatigue and its interaction with circadian rhythms. Adult rhesus monkeys -- trained to asymptote on a vigilance-discrimination (Vig-Disc) task and equipped to record circadian activity and temperature -- were fasted for 18 hrs before SP. During SP, successful performance was randomly reinforced with a food pellet 50% of the time, with trials every 2.4 min on average around-the-clock. This schedule produced continuous performance for at least 48 hrs. Performance lapses ranging from 2-15 min appeared during day 3 of SP, and performance lapses up to 72 min were seen in day 4 of SP. Despite lapses, monkeys performed on at least 86% of trials through hour 108 of SP. Performance degraded dramatically during SP. Vig was impaired 51.3% ($p<0.001$) and Disc 56.8% ($p<0.001$) during SP hrs 72-84. Circadian performance and physiology rhythms continued through SP, though fatigue altered both. These results show that we can use SP to study dramatic fatigue effects on performance and rhythms in monkeys. Supp by VA Res & USAMRDC.

519.20

DAYLIGHT SAVING TIME AND PSYCHIATRIC ILLNESS. C.M. Shapiro* (SPON: D. Apps). Dept. Psychiatry, University of Edinburgh, Edinburgh, EH10 5HF.

An improvement in mood after the spring adjustment of daylight saving time (DST) has been reported.

There are as yet no published accounts of the effect of DST on psychiatric illness.

Three groups were studied with regard to DST. In each case the analysis compared the week prior to the week following DST. The presentation of parasuicide to a regional poisons unit in Edinburgh (population 500,000) was considered. Over the years 1976-1986 there was no consistent pattern with regard to the spring or autumn DST change.

Admissions to the only psychiatric hospital serving the same population over a 16 year period were studied. All diagnosis entered in the Edinburgh Case Register were classified as having a possible affective diagnosis or not. There was no influence of DST in either group.

Information of all suicide deaths in Scotland over the period 1974-1983 was made available by the Scottish Home Office. An analysis of the date of death in relation to DST did not reveal any pattern either in the male or female subgroups.

In none of the three populations studied was there a discernible influence of DST. It may be that certain individuals are susceptible to small changes in circadian rhythm but this has not been reflected in the groups of this study with varying psychiatric morbidity.

INFECTIOUS DISEASES

520.1

TEMPORAL DEVELOPMENT OF THE BEHAVIORAL EFFECTS OF HERPES ENCEPHALITIS IN MICE. D. J. McFarland Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany NY 12201

The sequential development of the behavioral effects due to CNS infection with the herpes simplex type 1 virus was examined in two mouse models. Following intracerebral infection of adult female NYA/Nylar with the HF strain of herpes or adult female Balb/c mice with the F strain the majority of animals survived. An increase in motor activity observed 7 days following infection of NYA/Nylar mice coincided in time with a decline in brain virus titers as measured by a standard plaque assay. Likewise an increase in errors occurring during serial reversal performance in a water Y-maze was observed 8 days following infection of Balb/c mice and coincided with the declining phase of the viral growth curve. Taken together these results suggest that processes involved in the elimination of virus from brain, such as the cellular immune response, may be important in the development of the behavioral pathology produced by non-fatal herpes encephalitis.

520.2

EXAMINATION OF THE HSV INFECTION IN THE NEURON IN CULTURE C. L. Wilcox*[1], R. L. Smith*[2], E. M. Johnson, Jr.[3], and L. I. Pizer*[1], (SPON: M. Taniuchi) [1]Departments of Microbiology and Immunology, and [2]Pediatrics, University of Colorado Medical School, Denver, CO 80262, [3]Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

Herpes simplex virus (HSV) latency and viral gene expression was investigated in sympathetic neurons *in vitro*. Previously we showed that deprivation of nerve growth factor results in reactivation of latent HSV[1]. We found that inhibition of protein synthesis, pharmacological agents which elevate cyclic AMP, activation of protein kinase C with phorbol ester, and heat shock produced reactivation of latent virus.

HSV gene expression was examined during the productive infection, during latency and during the course of reactivation of latent virus in the neuronal cultures. Using conditions described previously[1] which result in the establishment of latent HSV infections in neuronal cultures, viral gene expression was below the limits of detection by dot blot analysis immediately after inoculation with virus, and at subsequent time points during the latent infection. Preliminary results using *in situ* hybridizations suggest that viral gene expression is limited during the latent infection, however the latency associated trasnscript was detected.

[1]J. Virology 62:393-399, 1988.

520.3

EFFECTS OF MUMPS VIRUS (MV) ON Na^+ AND Ca^{++} CHANNELS OF PC12 AND TE671 CELLS. E. K. Stauffer and R. J. Ziegler. *School of Medicine, Univ. Minn.-Duluth, Duluth, MN 55812

Persistent MV infection has been shown to reduce the excitable responsiveness of PC12 and TE671 cells (Ziegler & Stauffer, 1988. J.gen.Virol. 68:2501). The present study was designed to establish which electrogenic channels of PC12 and TE671 cells are altered by MV.

Stimulus-evoked action potentials (SEAPs) were recorded intracellularly from mock-infected (MI) and MV-infected cell cultures. Standard ionic channel blockers (tetrodotoxin, TTX; tetraethylammonium ion, TEA; and cobalt ion, Co^{++}) were used to differentiate and identify specific voltage-gated ionic currents/channels.

SEAPs recorded from MV cells without TTX were similar to those recorded from MI cells with TTX. Subsequent application of TTX to the MV cells had no significant effect. When MI and MV cells were examined while bathed in high Ca^{++} (20 mM), TTX and TEA, relatively small, slow rising SEAPs persisted in TE671 cells; in PC12 cells, SEAPs typical of Ca^{++} spikes for PC12 cells remained. In both cases, treatment with Co^{++} abolished the persistent SEAPs.

The results indicate that MV is exerting its effect via voltage-gated Na^+ channels. Ca^{++} channels appear not to be influenced by MV to any appreciable extent.

Supported in part by the Minnesota Medical Foundation and the Duluth Clinic Education and Research Foundation.

520.4

COGNITIVE-CEREBRAL MANIFESTATIONS OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) ENCEPHALOPATHY. R. Krikorian* (SPON: D. L. Garver). Dept. of Psychiatry, Univ. of Cincinnati Med. Ctr. Cincinnati, OH 45267-0559.

HIV has been shown to infect the nervous system, and, in brain, has been associated with cognitive and behavioral changes which can be independent of the clinical manifestations of other AIDS-related disorders. Patients at various stages of illness with serologic evidence of HIV infection and without signs of gross or focal neurological disorders were recruited for study. The subjects underwent comprehensive cognitive evaluations in order to characterize the nature of the intellectual decline associated with HIV infection.

A distinctive and consistent pattern of deficits was demonstrated, including impairments of attentional, organizational, and problem-solving abilities. Other abilities such as language, visual-spatial perception, and memory consolidation were relatively preserved. The deficits suggest compromise of a particular cerebral system composed of subcortical activating centers and projections to prefrontal cortex. This is consistent with evidence from neuropathology and neuroimaging studies indicating a predilection of HIV in brain for subcortical structures and white matter.

520.5

SEMI-QUANTITATIVE IMMUNOCYTOCHEMISTRY: A NEW METHOD FOR DETERMINING ALTERED PROTEIN PRODUCTION AS A CONSEQUENCE OF VIRAL INFECTION OR TOXIC AGENTS., F. J. Denaro, UCSD Med Ct, San Diego, CA

By use of double label histochemistry it has been possible to demonstrate that the neurosecretory cells of the adrenal medulla can be infected with Cytomegalovirus in the AIDS patient. While CMV infection can eventually lead to cell death, it is important to know if the quantity of biologically relevant proteins that the adrenal medulla produces can be altered during the course of infection. In order to test this possibility, a new double label method was developed. The PAP method was used to identify virus, while cell products (dopamine, chromogranin, synaptophysin, etc) were semi-quantified by radioimmunocytochemistry. This was done by use of a ^{35}S-streptaviden label (Amersham). It was then possible to compare grains between uninfected and infected cells. Toxins and other agents can alter cell products. Pentamadine has been found to be diabtogenic. A case was examined by semi-quantitative means in which insulin production was found to be significantly decreased, while NSE, S-100, and glucagon were unaffected. These results indicate that this semi-quantitative approach may be of value when it is necessary to compare relative amounts of cell products. Work is underway to apply this approach to transmitters.

520.6

QUANTITATION OF CEREBELLAR CHANGES IN RATS INFECTED WITH KILHAM RAT VIRUS. M.A. Littlefield-Chabaud, J.J. England+* G.F. Amborski++*, J.K. Daniloff Dept. of Anatomy and Fine Structure, Department of Microbiology and Parasitology++, LSU School of Veterinary Medicine, Louisiana Veterinary Medical Diagnostic Laboratory+, Baton Rouge, LA 70803-8408

Normal cerebellar histogenesis in the mammalian brain occurs in two epochs: although most neurons are generated early in utero, the granular cells divide and organize at or after birth. Most activity is complete by days 16-19. Kilham Rat Virus (KRV) of the Parvoviridae family has a predilection for rapidly dividing cells. The goal of this series of studies was to quantitate and describe in detail the direct effects of KRV on the developing cerebellum.

Five-day-old Sprague-Dawley rats (n=8) were injected intracerebrally with either 0.03 ml RPMI-1640 containing 300-500 plaque-forming units of KRV or 0.03 ml RPMI-1640. Cerebella were collected at post-injection days 10 and 30 and compared to normal control cerebella. Morphometric quantitation of the relative areas of each major region was performed (Bioquant System IV, Nashville, TN).

Preliminary results indicated the virus caused significant cerebellar hypoplasia at post-injection days 10 and 30 compared to normal and media-injected rats. Results will be compared with data on the normal development of the cerebellum and its interconnections. Supported by LA-SVM 862.

520.7

MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS IN THE CNS OF THE VIRALLY INFECTED RAT. D.L. Weinstein, D.G. Walker and P.L. McGeer, Kinsmen Laboratory, Dept. of Psychiatry, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada

The present study was designed to determine major histocompatibility complex (MHC) expression in rat CNS after intraneuronally introduced virus. Under anesthesia, rats received injections of 5 µl of Herpes simplex virus type I (HSVI) containing an estimated 27,500 PFU into the sclera of one orbit. On day 6, the animals were sacrificed and the brains fixed. Immunohistochemistry with monoclonal antibodies against rat MHC class I and class II antigens and polyclonal antibodies against HSVI and glial fibrillary acidic protein (GFAP) was carried out in both single and double staining procedures. Positive staining for HSVI was observed in the ipsilateral sensory nucleus of the fifth nerve as well as in punctate areas of the brainstem, thalamus and deep structures of the cerebellum. Most of the positively staining cells had the morphology of leukocytes although a few neurons were clearly outlined. In serial sections class II positive staining of non-neuronal cells was seen in areas positive for HSVI and did not overlap with GFAP-positive cells. The class II positive cells had the morphology of leukocytes, suggesting that, at these early time periods, CNS tissue was not expressing class II MHC antigens in response to the infection. (Supported by grants from the ADRDA and the MRC of Canada)

REFLEX FUNCTION: HUMAN I

521.1

LIMB ROTATION DEPRESSES SPINAL CORD REFLEX EXCITABILITY IN NORMAL HUMAN SUBJECTS. D.A. Lake. Dept Physical Therapy, Northeastern University, Boston, MA 02115.

Rhythmical rotation of the extremities is a common therapeutic technique used in the treatment of spasticity in stroke patients. The effects of limb rotation on the electrically evoked stretch reflex, H-reflex, were studied in 15 normal male and female subjects. Subjects were positioned supine on a low treatment table and asked to relax. Silver cup recording electrodes were placed on skin above the soleus. Monophasic (DC) square-wave pulses (0.2 ms duration, 1/sec frequency) were delivered to the tibial nerve in the popliteal fossa by a clinical bipolar electrode for identification of optimal stimulation sites. The clinical electrode was then replaced by two 1 inch square carbon impregnated rubber electrodes which were taped and straped to provide a stable source of stimulation while the lower limb was being rotated. The evoked responses were recorded onto magnetic tape for later analysis. H-reflexes were evoked prior to the beginning of rotation, 1, 2 and 3 minutes after rotation had commenced, and 30 sec, 1 and 2 minutes after the termination of rotation.

One minute of rotation of the lower extremity produced an average of 52% depression in the H-reflex when compared with control levels. This depression was maintained throughout the duration of rotation. Upon the cessation of the rotation, the H-reflex returned to control levels within 2 minutes.

521.2

EFFECTS OF POSTURAL AND STIMULUS VARIABLES ON HUMAN LIP-MUSCLE REFLEXES. M.D. McClean. Dept. of Speech Pathology, Univ. of Toronto, Toronto, Ont. M5G 1L4 CANADA

Lip muscles display early and late excitatory reflexes (E1 & E2) at approximate latencies of 15 and 35 ms, and suppression responses (S) at 20-50 ms. Parametric analysis of these reflexes may enhance understanding of their neural organization and lead to improved methods for clinical evaluation of the trigeminal-facial system. In the present study the pattern and magnitude of lip-muscle reflexes were investigated using independent stimulation of the upper and lower lips during lip rounding and lower-lip depression, and lateral stretch of both lips during a lip-press posture. Subjects maintained background EMG levels of 10-20% of maximum while 100 ms step displacements were delivered at the midline of the lips with a flat stimulus probe. Stimuli were applied randomly at five levels of acceleration with an electromagnetic shaker, and signal averages of lower-lip muscle EMG were analyzed. During lip-press and rounding postures prominent E1 responses were noted, and these showed strong positive correlations ($r > .9$) with stimulus magnitude. E2 responses were more variable in their occurrence across subjects. The magnitudes of E1 and E2 were greater for lower-lip compared to upper-lip stimulation. S responses were most evident during the lower-lip depression gesture and showed larger magnitudes to upper-lip compared to lower-lip stimulation. (Supported by the Hospital for Sick Children Foundation - Toronto)

521.3

COMPENSATION FOR MUSCLE YIELD AND LACK OF STIFFNESS REGULATION BY STRETCH REFLEXES. R.R. Carter*, P.E. Crago and M.W. Keith. Depts. of Biomedical Engineering and Orthopaedics, Case Western Reserve University, Cleveland, OH 44106.

The neuromuscular response to externally imposed adduction at the index finger metacarpal-phalangeal joint was recorded in normal subjects and decomposed into a passive component (measured with the muscle relaxed), an active mechanical component (estimated by electrically activating the first dorsal interosseus muscle) and a reflex component (calculated by subtracting the passive and active mechanical components from the total response). For large amplitude rotations, muscle yield was observed in the active mechanical component as an abrupt decrease in incremental stiffness to 22% to 70% of the small amplitude value. In contrast the total active stiffness (combined active mechanical and reflex stiffness) measured after onset of the reflex component differed from the pre-yield incremental stiffness by less than 9%. Thus, the reflex stiffness component of the stretch reflex provided excellent compensation for muscle yield. To investigate the stiffness regulation hypothesis, we employed a constant initial joint angle and rotational disturbance while modulating the initial torque level over the lower one-half of the available torque range. Both the mechanical component and reflex component increased with initial torque level, indicating that the reflex does not provide stiffness regulation.

521.4

MODULATION OF SOLEUS H-REFLEX EXCITABILITY WITH CHANGES IN HIP AND KNEE POSITION IN MAN. S.J. Sullivan, J. Pompura* and C.E. Chapman. Centre de recherche, Institut de réadaptation de Montréal; Concordia University; Université de Montréal, Montréal, CANADA.

The hypothesis that the facilitatory changes observed with controlled hip flexion in our previous studies were the result of afferent input resulting from stretch of the hamstrings group was investigated.

Soleus H-reflex recruitment curves were generated in 7 healthy subjects at one control (15° hip flexion) and two test positions: (1) hip flexion (40°) and (2) hip flexion (40°) plus knee flexion (36°). H-reflex testing followed a standard procedure (1ms pulse width, 0.1Hz). In position 1, Hmax increased in 3 subjects (10 - 26%), decreased in 1 (11%) and was unchanged in 3. In position 2, 4 subjects showed a marked decrease in Hmax (16 - 49%) and 1 showed an increase (18%). Only 1 of the subjects showing an increase in position 1 showed a decrease in position 2. The latter finding is not strongly supportive of the idea that hamstrings stretch contributed to the facilitatory effects of hip flexion alone on the soleus H-reflex. The pronounced inhibition seen in position 2 may be attributable to additional inhibitory inputs associated with the knee flexed position.

521.5

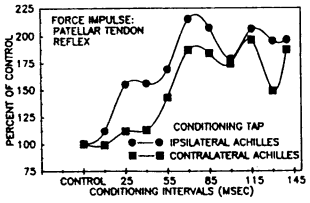
VIBRATION-INDUCED FACILITATION AND RECIPROCAL INHIBITION EFFECTS ON HUMAN SINGLE MOTOR UNIT CONTROL. J. Ives* and W. Kroll*(SPON: W. Abraham). Dept. of Exercise Science, Univ. of Massachusetts, Amherst, MA 01002.

The purpose of the present study was to assess changes in discrete volitional motor control when perturbed by facilitory and inhibitory reflexive input. Twelve (12) female subjects underwent standard single motor unit (SMU) control training of the tibialis anterior followed by two SMU control performance tests. EMG criteria measured were interspike interval (ISI), time to fire the SMU a specified number of times (TIME), number of extraneous motor unit potentials during SMU firing periods (ERRORS) and SMU rest periods (REST ERRORS). ISI and TIME were indices of specific SMU control while ERRORS and REST ERRORS measured ability to silence neighboring motor units. Treatment conditions consisted of applying tonic vibratory facilitation and reciprocal inhibition prior to performance testing. Stable baseline measures were taken on days 1 and 2 and treatment condition measures on days 3 and 4. The results from a 2-way repeated measures ANOVA revealed that reflexive facilitation increased ERRORS 433% and REST ERRORS 1638% ($p \leq .01$). The inhibition treatment increased ERRORS 388% ($p \leq .01$). Neither treatment significantly altered ISI or TIME. These results indicate that neither a facilitory or inhibitory reflex disrupted central activation of specific SMUs. The disruption of neighboring motor unit quiescence suggests that this inactivation is not due to central inhibitory command but relaxation of central activation.

521.6

PATELLAR TENDON REFLEX CHARACTERISTICS ARE ENHANCED BY A CONDITIONING ACHILLES TENDON REFLEX. D.M. Koceja*, J.M. Burke*, and G. Kamen. Motor Control Laboratory, Indiana University, Bloomington, IN. 47405.

Previous results in our lab demonstrated that a conditioning patellar tendon-tap reflex (PTR) produced a long-latency excitatory effect on the contralateral PTR. In this study, the PTR was conditioned by either an ipsilateral or a contralateral achilles tendon-tap stimulus in a group of normal college-age subjects (n=10). Paired tendon-tap reflexes were delivered with electromagnetic solenoids using conditioning intervals of 10, 25, 40, 55, 70, 85, 100, 115, 130 and 145 msec. Results indicated that a long-latency (> 50 msec) excitatory effect occurred for the PTR when conditioned by a tap to either the ipsilateral or contralateral achilles tendon, as shown for force impulse:



521.7

H-REFLEX MODULATION WITH THE GTO
M. Sabbahi, S. Olson*, M. Aldea*. Texas Woman's University School of Physical Therapy, 1130 M. D. Anderson Blvd., Houston, TX. 77030

The functional contribution of the Golgi Tendon Organs (GTO) in movement has been tested mainly in animal lower limb muscles. This study investigates the modulation of the H-reflex and presumably the motoneuron pool, during activation and deactivation of the GTO in flexor carpi radialis (FCR) muscles of human subjects. Normal subjects were tested using H-reflex of the FCR after electrical stimulation of the median nerve in the arm (0.2 pps, 1 MS at H-Max). Subject's hand was then loaded in extension using 2 pounds of weight and the peak-to-peak amplitude of the H-reflex was recorded. Two percent xylocain was then iontophoresed into the musculotendenous junction using 3 mA direct current for 20 min. H-reflexes were then recorded during unloaded and loaded conditions post anesthetic. Four H-reflexes were averaged at each phase and pre-test, post test comparison were carried out. Results showed that H-reflexes decreased by an average of 24% during loaded condition in 7 out of 8 subjects. This reflex inhibition was reversed after anesthetic was delivered to the GTO. During unloaded condition, H-reflex showed mild increase by an average of 8% in 5 subjects. Anesthetic iontophoresis resulted in substantial reduction of skin sensation under the electrode (2 cm diameter). Previous results showed >1 cm penetration of the anesthetic into the soft tissue using such treatment dose. These results imply that GTO of the flexor forearm muscles may cause inhibition of the homonymous motoneuron pool and this effect could be reversed by deactiviation using iontophoretic anesthesia. (Supported by NIH Grant #NS25297-01)

521.8

NONNOXIOUS SURAL NERVE STIMULATION EFFECTS ON TRICEPS SURAE HUMAN MOTOR UNITS. C.G. Kukulka, D.A. Brown* (SPON: C. Gisolfi). Physical Therapy Labs, The University of Iowa College of Medicine, Iowa City, IA 52242.

Experiments were done to evaluate the effects of nonnoxious electrical stimulation of the sural n on the discharging of human motor units from soleus (S) and lateral gastrocnemius (LG) muscles. Intramuscular motor unit recordings were made using bipolar fine wire electrodes. Single, 0.1 msec shocks were delivered at 3/s to the sural n at the ankle. Stimulation effects were evaluated by constructing poststimulus time and cumulative sum histograms. Stimulus intensities ranging from 1-5 times perception threshold (PT) were correlated with computer averaged evoked responses of the sural n. High linear correlations ($r \geq .09$) allowed representation of sural n input to the motoneuron pools in terms of PT as well as percentage of maximum evoked sural response. Complex excitatory/inhibitory changes were observed in 65 of 74 S spike trains and 13 of 15 LG trains. The predominant effects in S were inhibition-excitation-inhibition occurring between 38 and 137 msec poststimulation. Rarely (n=7), a short latency, mild excitation preceded this complex. In contrast to S, LG effects were of 2 types: a short latency inhibition between 40 and 60 msec (n=4) or inhibition-excitation between 70 and 110 msec (n=9). The results suggest that sural n afferent input may be disproportionately distributed to S and LG motoneurons in man. Supported by PHS Grant NS2499102

521.9

EVOCATION OF "EARLY" AND "LATE" COMPONENTS OF A CUTANEOUS REFLEX BY NON-PAINFUL STIMULI IN MAN. S. Nord and R. Durkovic. Neurol. Serv., VAMC; Depts. of Anat. and Physiol., SUNY Health Sci. Ctr., Syracuse, N.Y. 13210

It has been reported that the "early" (RII) component of a cutaneous reflex can be elicited by innocuous stimuli, whereas the "late" (RIII) component requires painful stimuli (e.g., Hugon, 1973; Willer, 1983). However, our preliminary experiments suggested that non-painful stimuli might produce both components. The present study focused upon this contradiction. Subjects were relaxed, normal adults. Surface EMGs were recorded via electrodes positioned over the short head of the biceps femoris muscle (SBi). Innocuous electrical stimuli were delivered in brief trains, one/min., to either the distal cutaneous distribution of the sural nerve or, percutaneously, to the retro-malleolar pathway of the sural nerve. Stimulation at each site generated SBi reflexes with both "early" and "late" components demonstrating that non-painful stimuli can elicit the "late" response under appropriate experimental conditions. Specifically, our data suggest that the low-threshold, longer latency component is consistently observed when intervals between stimulus presentations approach one min.. Notably, previous failures to observe this SBi response have occurred in studies which utilized much briefer interstimulus intervals (5-8s) but which were comparable to ours in other respects. Supported by NSF Grants BNS 8415917 and BNS 8808495.

521.10

EXTEROCEPTIVE REFLEXES IN MAN: STARTLING RESPONSES TO SHOCKING EXPERIENCES. R. Durkovic and S. Nord. Depts. of Physiol. and Anat., SUNY Health Sci. Ctr. and Neurol. Serv., VAMC, Syracuse, N.Y. 13210.

The present study was designed to survey the excitatory EMG reflex activity induced by the presentation of non-painful cutaneous or auditory (click) stimuli. Subjects were normal, relaxed humans in a supine position. Single clicks were delivered through padded earphones. Electrical stimuli, composed of brief trains of pulses, were delivered once each minute to various skin sites. Surface EMG recordings were obtained from electrodes overlying each of several different muscles.

Stimulation could induce both short and long latency reflex responses in muscles located relatively near the site of stimulation. In contrast, similar stimulation tended to trigger only the longer latency responses in muscles remote from the stimulation site. These findings are consistent with the following working hypothesis: "Early" reflex components have a local segmental organization, while "late" components result from suprasegmental activation organized in a pattern reminiscent of the "startle reflex" (e.g., Landis and Hunt, 1939). Supported by NSF Grants BNS 8415917 and BNS 8808495.

REFLEX FUNCTION: HUMAN II

522.1

THE ACTIVATION OF MOTOR UNITS IN REFLEX-INDUCED AND VOLUNTARY CONTRACTIONS IN HUMAN ARM MUSCLES. C.Gielen, E. van Zuylen*, J. Denier van der Gon*. Dept. of Medical and Physiol. Physics, Univ. of Utrecht, The Netherlands.

Motor-unit activity in human arm muscles was recorded with intramuscular electrodes during isometric contractions and during reflex activity elicited by torque perturbations in the elbow joint in flexion/extension and supination/pronation direction. Short latency responses to loading and unloading perturbations were found only in muscles that were stretched or shortened. However, changes in EMG activity at medium latency were also observed in muscles that were not stretched or shortened. Therefore, the medium latency reflex cannot be the result of a simple feedback mechanism that controls muscle length only.

During medium-latency reflex activity different types of motor-units were found in nearly all muscles, each with a different type of activation. The relative activation of these subpopulations in the medium latency reflex for torques in different directions counteracting the perturbation was the same as that observed during voluntary contractions in corresponding directions. The results demonstrate that the medium latency reflex reflects the coordinated activation of muscles which is necessary for an adequate response. The similar activation of motor units for voluntary and reflex-induced contractions suggests that the activation in both conditions is mediated by the same coordination center.

522.2

HUMAN FLEXOR REFLEX REVERSAL IN KNEE MUSCLES DURING CYCLING. D.A. Brown*, C.G. Kukulka, T. Cook*. Physical Therapy Education, The University of Iowa, Iowa City, IA 52242.

The human flexor reflex (HFR) in lower extremity muscles has been studied almost exclusively during static resting and static isometric conditions. In an attempt to characterize this reflex during a locomotive movement, subjects cycled at a constant workload while an electrical stimulus was delivered to the tibial nerve at the medial malleolus (0.1 ms rectangular pulses at 300/s for 25 ms). The EMG responses recorded in rectus femoris (RF) and biceps femoris (BF) at various phases of knee motion were compared to the mean EMG regularly occurring at these intervals with no stimulus present. Results indicate a pattern of inhibition-excitation-inhibition in RF during knee extension phases, and an excitation-inhibition-excitation pattern in BF during knee flexion. The peak amplitude of the excitatory responses are dependent on both the mean baseline EMG activity and the knee angle at which stimulation occurs. The observed patterns strongly support previous studies investigating phase dependent reflex reversal in animal and human subjects.

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522.3

EVIDENCE FOR PHASE DEPENDENT CUTANEOUS REFLEX REVERSAL DURING WALKING IN HUMANS. J. F. Yang, R. B. Stein, Department of Physiology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Phase dependent cutaneous reflex reversals in walking have been reported for some time in cats, but never demonstrated in humans. This study examines these responses in humans. Twin pulses of approximately four times sensory threshold were applied randomly to the tibial nerve, posterior to the medial malleolus, where the nerve is mostly cutaneous. An M-wave recorded from the abductor hallucis served as control. Responses were recorded from the ipsilateral tibialis anterior (TA), soleus (SO) and rectus femoris (RF). The walking cycle was divided into 16 parts; stimuli which occurred within one part were averaged together. The most prominent and consistent responses occurred at a latency of 70 to 90 ms in an active muscle. The responses were not observed in an inactive muscle. In the TA and RF muscles which are active in both the stance and swing phase of walking, a clear reversal in the direction of the response was observed: inhibition in the stance phase, excitation in the swing phase. These results suggest that a functionally appropriate reversal in the cutaneous responses does occur in the walking human.
(Supported by MRC and AHFMR)

522.4

CONSTANT ERRORS IN HUMAN FORCE ARE PREDICTED BY CONTRACTION-INDUCED PLASTICITIES IN THE STRETCH REFLEX. R.S. Hutton, A.C. Powers*, H.K. Kelsh* and S. Suzuki*. Dept. of Psychology, Univ. of Washington, Seattle, WA 98195.

Conditioning agonist muscle contractions cause post-contraction afterdischarge and increased stretch sensitivity in muscle spindle receptors. Under static conditions, the tonic stretch reflex is enhanced leading to non-volitional increments in muscle activity. Predictably, when human subjects estimate a previously learned criterion force (LCF) following a conditioning contraction, estimates of the LCF are overestimated compared to control values. The amplitude of these constant errors decreases over time as does the intensity of muscle spindle afterdischarge, and both are attenuated by muscle stretch (Hutton et al., J. Physiol., 393, 247-259, 1987). Since Ia afferents synapse with Ia reciprocal inhibitory interneurons, a maximum elbow extensor force (5 s duration) preceding 35 s estimates of a flexor LCF (2% max.) should induce underestimates in force due to afterdischarge-linked reciprocal inhibition from the extensors. This prediction was tested in 25 human subjects. Following an extensor conditioning force, estimates of the flexor LCF were consistently underestimated. The size of the underestimate was time dependent. Previous findings of an overestimate of a flexor LCF following a maximum flexor contraction were replicated. The direction of constant errors in human force can therefore be explained by known contraction-induced plasticities in muscle spindles.

522.5

VIBRATION-EVOKED RECIPROCAL INHIBITION BETWEEN HUMAN WRIST FLEXORS AND EXTENSORS. F.W.J. Cody* and T. Plant* (SPON: A.M. Smith) Dept. of Physiol. Sci., Univ. of Manchester, Manchester M13 9PT England.

Vibration stimuli (1, 2, 3 or 19 cycles, 0.7-1.3mm amplitude, 125Hz) were applied to the tendon of flexor carpi radialis (FCR) to evoke Gp Ia-dominated afferent discharges whilst the resulting reciprocal inhibition of its antagonist (extensor carpi radialis, ECR) was quantified as a reduction of asynchronous, on-going e.m.g. The protocol conformed to the Declaration of Helsinki.

The most marked and repeatable response, evident in 90% of trials from 16 subjects, was a reduction in ECR rectified e.m.g. activity commencing at about 40ms, lasting some 20ms and reaching a minimum of approximately 55% of the pre-stimulus level. In about 25% of trials a shorter latency (25ms), smaller (minimum of 70% pre-existing level) depression of e.m.g. was present. The amplitudes of both phases of inhibition increased in proportion to background e.m.g. for voluntary ECR contractions of 10-30% maximum. Neither phase was appreciably affected by changes in stimulus duration. Comparable patterns of reciprocal inhibition of FCR were observed, in 6 subjects, upon vibration of the belly of ECR.

Our findings, in the wrist musculature, of short-latency, reciprocal inhibition, probably mediated by Gp Ia, oligo-synaptic, spinal reflex action, and a later, more pronounced reduction in motor discharge agree with earlier descriptions from H-reflex studies (Cavallari, P. et al., Exp. Brain Res., 56, 574, 1984; Day, B.L. et al., J. Physiol., 349, 519, 1984). Two important differences were, however, noted:- (i) the overall duration of inhibition was far shorter and (ii) reciprocal inhibition increased, rather than decreased, during voluntary activation of the inhibited muscle.

522.6

MODULATION OF H-REFLEX FOLLOWING SURFACE SKIN ELECTRICAL STIMULATION, XYLOCAINE AND PLACEBO ANESTHESIA IN CONTROL SUBJECTS. A.Y. Belanger, A.B. Arsenault, M.J. Durand* and L. Fortin*. Research Center, Montreal Rehabilitation Institute, Montreal, Canada. H3S 2J4

The purpose of this study was to determine, in 12 subjects, the extent of soleus motoneuron excitability during conditions of increased (Electrical Stimulation, ES), decreased (Xylocaine Anesthesia, XA) and normal (Placebo Anesthesia, PA) cutaneous inputs. Skin ES was applied using a TENS unit, with the two pairs of electrodes placed respectively over the Achilles (S1 dermatome) and TA (L5) tendons. True and placebo anesthesia were respectively obtained after rubbing some Xylocaine (5%) and Vaseline ointment on the skin surface overlying the Achilles tendon. Sets of 10 H-reflexes (Hmax/2) were evoked (1 shock/30s) and averaged at different time intervals before, during and after the testing conditions (Hmax values were stabled throughout testing). The results, apart from showing a small (10%) H-reflex facilitation during Achilles tendon ES, revealed PA to cause the same gradual facilitatory response (up to 100% after 50 minutes) as that obtained during XA. This finding would appear to seriously challenge the view that reducing (via true anesthesia) neural activity from the skin to the soleus motoneurons significantly increases their excitability. We postulate that the elicitation of consecutive H-reflexes per-se (1 every 30s for minutes) facilitated the H-reflex over time.
Funding: Montreal and Laval University, and Medtronic Inc.

522.7

DILUTE NOVOCAIN BLOCK OF THE STRETCH REFLEX IN HUMANS DURING STRETCH-SHORTENING CONTRACTIONS. H.A. Kilani[1]*, S.S. Palmer, M.J. Adrian*, and J.J. Gapsis[2]*. [1]Faculty of P.E., Univ. of Jordan; Dept. of Kinesiol.,[2]Div. of Rehab. Educ.Servs., Univ. of Illinois at Urbana-Champaign,IL 61801

Intramuscular injection of 1% Novocain (4-10cc) was used to block the stretch reflex of vastus lateralis (VL) to investigate stretch reflex contribution to stretch-shortening contractions with either tonic or dynamic pre-stretch. Six male athletes performed squat-jump relaxed (SJR, from seated on a chair), squat-jump tension (SJT, from knee flexion), squat-jump hop (SJH, on one leg), counter-movement jump (CMJ, with fast prestretch), counter-movement hop (CMH, on one leg), and drop jump (DJ, from 50 cm high box) trials before and after injection. Ground reaction forces were measured; EMG from VL was rectified and integrated (IEMG). Ten IEMG responses to tendon tap were averaged before and after injection. Reflex amplitude decreased 52-87%. Repeated measure ANOVA was used to analyze jump height (H), maximum force, IEMG, and vertical velocity (V). Since no change occurred in SJR variables, muscle function without prestretch was not compromised. Significant decreases occurred in both tonic /H - SJH(7%); V - SJH(5%)/ and dynamic /H - CMH(13%), DJ(10%); V - CMH (7%); IEMG - CMJ(20%), CMH(25%)/ prestretch conditions. Two leg tests may include some contralateral leg compensation. The stretch reflex contributes more to enhanced jump height during dynamic prestretch than tonic pre-stretch.

522.8

DEVELOPMENTAL AND AGING ASPECTS OF HUMAN ORAL-MOTOR REFLEXES. C.M. Weber, A. Smith, M. Denny*, and J. Newton*. Dept. Audiology and Speech Sciences, Purdue University., W. Lafayette, IN 47907.

The activity of human jaw-closing muscles is extremely sensitive to modulation by sensory feedback arising from low-threshold intraoral mechanoreceptors, and reflex responses of jaw-closing muscles elicited by mechanical stimulation of sites on the palate and tongue show a striking spatial organization (Smith et al., Exp. Neurol., 90:489-509). The aim of the present investigation was to explore the influence of developmental and aging processes on the nature of these reflex responses previously described for young adults. The patterns, latencies, and amplitudes of oral-motor reflex responses of children (7-8 years) and elderly adults (70-80 years) were examined.

Reflex responses in jaw-closing muscles were elicited with innocuous mechanical stimuli applied to sites on the palate and tongue dorsum. During stimulation, subjects maintained a constant isometric biting force. Reflex responses were measured as changes in biting force and in activity recorded from bilateral masseter muscles.

The complexity in patterns and spatial organization of oral-motor reflex responses elicited in children and elderly subjects were very similar to those previously described for young adults. However, there do appear to be subtle age-related effects on patterns, latencies, and amplitudes of responses for given stimulation sites.

522.9

TENDON TAP REFLEX RESPONSES AS INFLUENCED BY MOVEMENT IMPAIRMENT. N.J. Lambert*, W.P. Kroll*, and L.D. Abraham. Motor Control Lab, Univ. of Mass., Amherst, MA 01003.

Patellar tendon tap response to a maximum tap force were compared in an able-bodied group (AB,n=10) and a spastic group (SP,n=10) with respect to EMG latencies, peak to peak amplitude (AMP) of the largest spike, and muscle force (FORCE). SP subjects consisted of spinal cord injured with no voluntary movement (SCN,n=3) and impaired movement (SCM,n=3), or with cerebral palsy and impaired movement (CPM,n=4). Latencies from the tendon tap to the appearance of the muscle action potential (INITIAL), to the first peak of the largest spike (FIRST), and to the second peak of the largest spike (SECOND), were stable over six trials on each of two test days as indicated by intraclass R's of greater than .90. Latency differences were observed between the groups for INITIAL ($p<.01$; AB, x=21.4 msec; SP, x=18.6 msec) and FIRST ($p<.05$; AB, x=29.2 msec; SP, x=23.8 msec) measures. Latency differences may in part be due to differences in limb length. AMP and FORCE measures were also stable with intraclass R's of greater than .86. Differences between groups in AMP ($p<.01$; AB, x=267 uv; SP, x=786 uv) and FORCE ($p<.05$: AB, x=16.7 N; SP, x= 33.0 N) were also observed. Responses were largest for the SP subjects with impaired voluntary movement for AMP (CPM, x=1478 uv; SCM, x=741 uv; SCN, x=264 uv), and FORCE (CPM, x = 43.4 N; SCM, x=38.0 N; SCN, x=15.3 N) measures. The latter results indicate that the greatest spasticity occurs in subjects with impaired movement.

522.10

RELATION BETWEEN PROBE CONTACTOR CONFIGURATION AND THE MECHANICALLY EVOKED PERIORAL REFLEX. S.M. Barlow, Speech-Orofacial Physiology Laboratory, Boys Town National Institute, Omaha, NE 68131.

Recent evidence suggests that execution of fine motor behaviors by orofacial structures is dependent on peripheral inputs originating from a number of mechanoreceptors located within skin and muscle. These studies also have shown that a number of factors may influence the magnitude and distribution of afferent mediated processes, including the nature of peripheral input.

The purpose of the present investigation was to characterize the reflex sensitivity of perioral muscles to mechanical stimulation involving a series of contactor probes varying in size and configuration. A linear motor was used to deliver taps, with displacements ranging from 50 to 1500 microns, to hairy and glabrous skin of the lower face in humans. Several types of electrodes were used, including a highly selective intramuscular 2-conductor hook-wire for motor unit recordings, and 80 micron intramuscular hook-wire and miniature Ag/AgCl surface disks for recording gross EMG. Recording sites in the lower face included the medial and lateral segments of the orbicularis oris inferior, orbicularis oris superior, and mentalis. Young adult subjects were coupled to a specially designed transducer that allowed real-time monitoring of lip force during the presentation of mechanical stimuli to perioral skin. This was a necessary control since motoneuron activity (facial nucleus) is related to reflex magnitude. Preliminary results suggest that the threshold of the early component of the perioral reflex (14 to 20 millisecond latency) is inversely related to the probe contactor array size. Moreover, the distribution of the mechanically evoked perioral response is generally ipsilateral and confined to the muscles proximal to the stimulated skin.
(Supported by NIH grants NS-19624-05 and NS-23825-02)

523.1

DESENSITIZATION OF THE ALPHA-2 ADRENERGIC RECEPTOR IN HT29 CELLS. S.B. Jones* and D.B. Bylund (SPON: J.L. Lewis), Department of Pharmacology, Univ. of Missouri, Columbia, MO 65212.

The alpha-2 adrenergic receptor is coupled in an inhibitory manner to adenylate cyclase. Preincubation with an alpha-2 adrenergic agonist would be expected to result in an attenuation of inhibition upon a subsequent exposure of cells to that agonist. HT29 human colon adenocarcinoma cells have alpha-2 adrenergic receptors which are negatively coupled to adenylate cyclase. Utilizing the [^{3}H]adenine prelabelling technique, we preincubated cells for 30 min with norepinephrine and then stimulated with forskolin and various concentrations of UK14,304, an alpha-2 agonist. Preincubation with norepinephrine resulted in a rightward shift in the dose response curve to UK14,304 resulting in a 20-fold increase in the EC_{50} for UK14,304 (3 to 60 nM). Because preincubation of HT29 cells with norepinephrine results in a 10-fold increase in forskolin-stimulated cyclic AMP production, we repeated the experiment utilizing VIP in the stimulation phase of the assay instead of forskolin. Norepinephrine preincubation again caused a dose-dependent rightward shift in the dose response curve to UK14,304 indicating desensitization of the alpha-2 adrenergic receptor in HT29 cells. (Supported by NIH GM37664.)

523.2

ALPHA$_2$-ADRENERGIC RECEPTORS DOWN-REGULATION BY ALPRAZOLAM. Tyrone Lee. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada M5T 1R8.

Recent reports have shown that alprazolam, a triazolobenzodiazepine, is effective in treating depressed patients with potency equivalent to that of other tricyclic antidepressants. In our earlier attempt to screen for drug action on various receptors in the CNS (Lee et al., Soc. Neurosci. Abst. 12:1080, 1986), alprazolam was found to reduce α_2-adrenergic receptor density. The present report investigates the dose-effect relationship of this action.

Six groups of male Wistar rats of 12 each were injected with daily doses of either saline (1 ml/rat) or desipramine (5 mg/kg) or alprazolam (1, 2.5, 5, 10 mg/kg) for 21 days. Brain α_2-adrenergic receptor density was determined by Scatchard analysis using ^{3}H-clonidine and phentolamine. The results are as follows:

	Bmax (fmoles/mg protein)	% Change
Saline Control	159 ± 6	0
Desipramine (5 mg)	142 ± 4*	-11
Alprazolam (1 mg)	141 ± 5@	-12
(2.5 mg)	131 ± 5¢	-18
(5 mg)	139 ± 5*	-13
(10 mg)	140 ± 4*	-12

¢ = $P < 0.01$; * = $P < 0.02$; @ = $P < 0.05$.

It is concluded that alprazolam significantly reduced the density of the α_2-adrenergic receptors at all doses attempted in this study. (Supported by the Clarke Institute Research Fund.)

523.3

CYCLIC AMP AND ADENOSINE DIFFERENTIALLY REGULATE THE EXPRESSION OF ALPHA-1 RECEPTORS IN DDT-1 CELLS. J.B. Schachter and B.B. Wolfe. U. PA Sch. Med., Phila. PA 19104.

We have observed that increases in [cAMP] induce an increase in the number of alpha-1 adrenergic receptors (α_1-R) in the DDT-1 smooth muscle cell line. α_1-R number, measured by the binding of ^{3}H-prazosin, reached 30 to 80% above control after 20 hours of drug treatment. The appearance of new receptors correlated with an increase in α_1-R stimulated turnover of inositol phosphates (PI).

Adenosine can inhibit cAMP production via A_1 receptors. We have observed that A_1 agonists downregulate α_1-R in DDT-1 cells by 20 to 30%. Conversely, A_1 antagonists upregulate α_1-R by 20%, suggesting that endogenous adenosine tonically downregulates α_1-R.

Since cAMP upregulates α_1-R and since A_1 receptors are negatively coupled to cAMP production adenosine could have been downregulating α_1-R by decreasing the basal [cAMP]. However, downregulation of α_1-R by adenosine agonists still occurred in the presence of 8-bromo cAMP. As an alternative mechanism, acute administration of A_1 agonists potentiated PI turnover in these cells. Thus, although A_1 receptors do inhibit cAMP production and cAMP does regulate α_1-R number, adenosine may heterologously downregulate α_1-R by enhancing the activity of a feedback mechanism involving PI and diacylglycerol. Supported by GM31155.

523.4

ESTROGENS MODULATE STIMULATION OF PHOSPHOINOSITIDE HYDROLYSIS BY NOREPINEPHRINE (NE) IN RAT BRAIN SLICES. A. Favit*, L. Fiore* and P.L. Canonico (SPON: C. Bethea). Institute of Pharmacology, Catania Univ. Sch. of Med., Catania, Italy.

Estrogen treatment modified stimulation of inositol phospholipid (PI) hydrolysis in slices prepared from rat hippocampus, corpus striatum and cerebral cortex. This effect was sex-dependent and required repeated injections of the steroid. In ovariectomized female rats, a 7-10 day treatment with estradiol benzoate (2 ug/animal subcutaneously, once every two days) [^{3}H]inositol monophosphate [^{3}H]InsP production in response to NE in hippocampal slices, while an increased responsiveness was found in the cerebral cortex. No effect was observed after a single injection of estradiol benzoate or after in vitro addition of 17-beta-estradiol to brain slices. In chronically treated male rats, the PI response to NE was increased in the hippocampus with no change in the cerebral cortex. The most dramatic changes induced by repeated estrogen treatment were observed in the corpus striatum, where the stimulation of [^{3}H]InsP production by NE was drastically reduced in both sexes. We are currently investigating whether this effect of estrogens at striatal level may be correlated to the antidyskinetic properties of the steroid.

523.5

MELATONIN'S INTERACTION WITH β-RECEPTORS IN RAT PINEAL. T. Warcholak* and L.P. Niles (SPON: G.M. Brown). Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

Binding studies in our lab have suggested an interaction between norepinephrine and [^{3}H]melatonin binding sites in rat hippocampus (1). In order to clarify this issue, the effects of melatonin on the binding of the β-adrenergic antagonists, [^{125}I]iodocyanopindolol ([^{125}I]CYP) and [^{3}H]dihydroalprenolol ([^{3}H]DHA), were examined.

Saturation studies indicate that melatonin does not alter [^{3}H]DHA binding in rat brain. When incubated with homogenates from rat pineal, melatonin enhanced [^{125}I]CYP binding up to 50% above control values. This effect was dose-dependent ($EC_{50} \approx 5 \times 10^{-9}$ M), in agreement with previous studies in rabbit pineal (2). Preliminary findings suggest that 100 nM melatonin significantly enhances the affinity of β-adrenergic sites in rat pineal. Control: K_D = .20 nM, Bmax = 1090 fmol/mg protein; Melatonin: Kd = .11 nM; Bmax = 930 fmol/mg protein.

Additional studies are required to confirm these findings and to determine the possible mechanism(s) involved in mediating melatonin's effects.

1. Niles, L.P.: J. Pineal Res., 4, 89-98, 1987.
2. Sweat, F.W.: Biochem. Biophys. Res. Comm., 138, 1196-1202, 1986.

523.6

BETA-2-ADRENOCEPTOR SELECTIVITY OF A PROBABLE IRREVERSIBLE CARBOSTYRIL-BASED BETA-AGONIST. S.P. Baker, J. Pitha*, P. Posner* and K.M. Standifer. Depts. of Pharmacology and Physiology, Univ. of Florida, College of Med., Gainesville, FL 32610 and GRC-National Institute on Aging, Baltimore, MD 21224.

Previous studies suggest that a bromoacetamido congener of 8-hydroxycarbostyril (C-Br) is a potent beta-agonist which may bind to the beta-adrenoceptor in an irreversible manner. In the present study, the effects of *in vivo* administration of C-Br on the beta-adrenoceptor of several tissues was determined. Similar to isoproterenol, C-Br (0.1 and 5 mg/kg) stimulated rat heart and lung ornithine decarboxylase activity which was blocked by propranolol. Three hours after an i.p. or s.c. injection of C-Br (0.5-10 mg/kg), there was a dose-dependent loss of beta-adrenoceptors in the lung and spleen, the maximal loss being in the range of 75-85%. In contrast, the maximal loss of receptors in the heart and submaxillary gland was 15-25% and no receptor loss was detected in the cerebral cortex even at the highest dose. Competition assays using [^{125}I]iodocyanopindolol and the highly selective beta-antagonist CGP-20712A showed that the majority of receptors in the heart and submaxillary gland are beta-1 whereas the majority in the lung and spleen are beta-2. After treatment with C-Br (5 mg/kg), the competition assays showed that the loss of receptors in the 4 tissues was mainly of the beta-2 subtype. The selectivity of receptor loss was further confirmed by C-Br pretreatment of isolated membrane preparations from the tissues. The data suggest that C-Br is a beta-agonist *in vivo* with a high degree of irreversible selectivity for the beta-2 subtype. In addition, C-Br may not be able to cross the blood brain barrier. Supported by NIH grants HL32099 and GM34905.

523.7

DESENSITIZATION OF THE BETA-ADRENERGIC SYSTEM WITH AN IRREVERSIBLE AGONIST IN DDT_1-MF_2 CELLS. K.M. Standifer, J. Pitha*, and S.P. Baker. Dept. of Pharmacology, University of Florida College of Medicine, Gainesville, FL 32610, and GRC-National Institute On Aging, Baltimore, MD 21224.

We recently reported on the characterization of a bromoacetamido congener of 8-hydroxycarbostyril (C-Br). Our results showed the compound to be a beta-agonist that may bind irreversibly and produced sustained activation effects *in vitro* (Soc. Neurosci. Abstr. 13:148, 1987). In the present study, the effects of C-Br were investigated using the intact cultured cell system DDT_1-MF_2. Competitive binding studies using [^{125}I]iodocyanopindolol (ICYP) in the presence of 100 µM Gpp(NH)p showed that C-Br was 16 times more potent than (-)isoproterenol (iso). Measurements of cAMP accumulation in intact cells revealed that C-Br is 9 times more potent than iso, but equally efficacious. A time course of cAMP accumulation revealed that both iso and C-Br induced a cAMP peak level at 3 min followed by a similar decline, which nears basal levels by 60 min. Addition of propranolol at 3 min resulted in a rapid drop of cAMP to basal levels in the iso-induced system, but had no effect on the C-Br induced one. A time course of receptor loss with 10 µM iso or 1 µM C-Br treatment of intact cells was measured with [^{3}H]CGP-12177. Both iso and C-Br induce a time-dependent loss of receptors as measured on the cell surface. Cells assayed 24 hr later revealed that binding sites from iso treated cells had nearly returned to control levels, whereas the C-Br treated cells still showed an 80% loss of sites.

These results imply that C-Br, like iso, causes a desensitization in intact cells. Treatment of cells with iso for 60 min causes a redistribution of receptors, while treatment with C-Br results in loss of binding sites that has not reversed by 24 hr.

523.8

REGULATION OF SUBTYPES OF CENTRAL BETA ADRENOCEPTORS (BARs) AFTER CHRONIC INTRACEREBROVENTRICULAR INFUSION OF ISOPROTERENOL IN RATS **C. Gambarana*, G.A. Ordway*, & A.Frazer (Spon., D. Brunswick). V.A. Med. Ctr. & Univ. of Pa. Sch. of Med., Phila., PA 19104.**

Isoproterenol (ISO) causes a time-dependent reduction in the density of either beta-1 or beta-2 receptors when added to tissue preparations in vitro. To determine whether a similar phenomenon would occur in vivo with brain BARs, ISO was infused into the lateral ventricle of rats (300g) for 7 days at doses of either 5 ug or 15 ug/hr. Control rats received infusions of 0.9% NaCl. These infusions were done in rats treated at birth with either 6-hydroxydopamine (6-OHDA) or saline (sham-lesioned). Subtypes of BARs were measured in 23 regions by quantitative autoradiography (Proc. Natl. Acad. Sci. 81:1585, 1984). Neonatal treatment with 6-OHDA caused a significant increase of beta-1 receptors in 16 regions and a significant elevation of beta-2 receptors in 14 areas. In sham-lesioned rats ISO reduced significantly the binding of ^{125}I-Iodopindolol (IPIN) to beta-2 receptors in more regions (16) than the binding of the ligand to beta-1 receptors (2 regions). A similar result occured in the 6-OHDA treated rats as ISO reduced binding of the ligand to beta-2 receptors in 16 areas and to beta-1 receptors in 8 regions. The magnitude of the reduction that ISO caused in beta-2 receptors was greater than the reduction in beta-1 receptors in sham-lesioned rats and this tended to occur also in 6-OHDA lesioned rats. Even in rats lesioned with 6-OHDA, chronic infusion of ISO has a greater effect on the density of central beta-2 than on beta-1 receptors. **(Supported by Research Funds from the VA and USPHS grant MH29094).**

523.9

DMI TREATMENT DOES NOT DECREASE NE STIMULATED ADENYLATE CYCLASE ACTIVITY IN RAT CORTICAL SLICES FROM LONG-TERM NE DEPLETED ANIMALS. M.W. Dudley and B. Baron. Merrell Dow Research Institute, Cincinnati, OH 45215

β-Receptor (β-rec) density in cortical membranes and cAMP production in cortical slices were measured 35 days after treatment with xylamine (XYL). XYL (20 mg/kg) produced a sustained selective ≈50% decrease in cortical NE, DHPG and MHPG. Presynaptic markers indicated no loss of neuronal integrity or number. Dose-response curves with the non-selective agonist NE on cAMP formation showed the loss of NE antagonized the normal desipramine (DMI) induced (10 mg/kg, days 21-34) decrease in maximum response. The inclusion of prazosin (1 µM) or phentolamine (5 µM) in the NE dose-response study in these same tissues resulted in the expected decrease in maximal cAMP levels. Neither prazosin nor phentolamine had an effect on the NE dose response in control or the NE-depleted animals. The loss of NE did not affect the number or the selective agonist affinity for α_1- or α_2-receptors. The loss of NE did not effect basal β-rec number or the dose response to stimulation of cAMP formation by the selective β-agonist isoproterenol (ISO). DMI significantly decreased β-rec number and the maximum response to ISO on cAMP formation in control and NE-depleted animals. Recently published data has shown that central α-receptors can modulate β-rec adenylate cyclase activity. A chronic NE deficit may effect the coupling of α-receptors to their second messenger systems which may be important in the postsynaptic effect of antidepressant treatment.

523.10

ONTOGENY AND CHRONIC ANTIDEPRESSANT REGULATION OF β_1- AND β_2-ADRENERGIC RECEPTOR MESSENGER RNA IN RAT BRAIN. R.S. Duman and J.F. Tallman, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Previous studies have characterized the ontogeny and chronic antidepressant regulation of ß-adrenergic receptors (βAR) by ligand binding analysis. However, little is known about βAR regulation at the level of gene transcription and translation. In the present study we examine mRNA levels for both the β_1AR and β_2AR subtypes in rat brain by hybridization blot analysis using cDNA clones specific for each adrenergic receptor subtype.

During early postnatal development (days 1-7) the density of βAR binding sites is very low (< 25% of adult) but then increases to adult levels by day 16. β_1AR and β_2AR mRNA levels exhibit a similar pattern of development except that β_1AR mRNA is expressed at relatively higher levels than β_2AR mRNA during the early time points. These results suggest that βAR receptor binding sites during days 1-7 are primarily of the β_1AR subtype.

Chronic imipramine administration (2-3 weeks) results in a decrease in the number of β_1AR but not β_2AR binding sites. Preliminary studies indicate that chronic imipramine treatment influences mRNA levels of both βAR subtypes; in contrast to the decrease or no change in binding sites, both β_1AR and β_2AR mRNA levels are increased by this treatment. The results suggest that chronic antidepressant treatment increases the synthesis and turnover of βAR binding sites or blocks the translation and expression of functional receptor binding proteins.

523.11

ELECTROCONVULSIVE SHOCK (ECS) DECREASES BETA-ADRENERGIC RECEPTOR BINDING DESPITE SEROTONIN LESIONS. C.A. Stockmeier and K.J. Kellar. Department of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, OH 44106; Department of Pharmacology, Georgetown University School of Medicine, Washington, DC 20007.

Repeated daily administration of ECS causes a decrease in the number of antagonist-labelled beta-adrenergic receptors in rat cortex (Bergstrom and Kellar, 1979). It has been proposed that serotonin axons play a critical role in this process (Nimgaonkar et al., 1985). We examined the influence of 10 days of repeated daily ECS on [^{3}H]dihydroalprenolol binding sites for which isoproterenol has high and low affinity in control rats and in rats which received an injection of 5,7-dihydroxytryptamine (5,7-DHT) into the dorsal and median raphe nuclei. In competition experiments in cortex, ECS decreased the number of high-affinity binding sites, but did not alter the K_i of the high- or low-affinity binding sites or the number of low-affinity sites. Extensive lesions of serotonin neurons with 5,7-DHT resulted in a large increase in the number of low-affinity binding sites. Repeated ECS still resulted in a decrease in the number of high-affinity binding sites in rats with lesions of serotonin neurons, and the decrease was similar to that seen in rats without lesions. Hence, ECS does not require intact serotonin neurons to decrease the number of high-affinity beta-adrenergic receptors in rat cortex. Supported by NS24523, MH41819 and MH41684.

523.12

PROLONGED ELECTROSHOCK CHANGES THE COUPLING BETWEEN α- AND β-ADRENOCEPTORS IN RAT BRAIN. A. Pilc, I. Nalepa*, J. Vetulani* and S.J. Enna[1]. Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland and [1]Nova Pharmaceutical Corp., Baltimore, Md. 21224.

The cyclic AMP response to β-adrenergic agonists is greatly enhanced by α-adrenergic stimulants suggesting a functional interrelationship between these receptor systems in brain. Chronic administration of antidepressants diminishes this receptor interaction, indicating an association with the response to this drug class (Pilc, A. and Enna, S.J., Life Sciences 37: 1183, 1985). The present study was undertaken to examine whether electroshock, the most efficacious antidepressant therapy, modifies this coupling as well. For the study, rats were exposed to electroshock once daily for 7 consecutive days after which cyclic AMP and inositol phosphate (IP) accumulation were examined in rat brain cerebral cortical slices using prelabeling techniques. Whereas electroshock treatment had no effect on isoproterenol-stimulated second messenger production or on norepinephrine-stimulated IP accumulation, it significantly reduced the cyclic AMP response to norepinephrine alone and to isoproterenol in the presence of 6-fluoronorepinephrine, an α-adrenoceptor agonist. The results suggest that antidepressants modify the functional coupling between β-adrenoceptors and an α-adrenergic site distinct from that associated with inositol phosphate production.

523.13

HUMAN LYMPHOCYTE BETA ADRENERGIC RECEPTOR DENSITY - POSSIBLE RELATIONSHIP TO PANIC-AGORAPHOBIC SYMPTOMS IN DEPRESSED WOMEN.

J. Magliozzi, D.W. Gietzen, R. Maddock* and A. Doran*. Dept. Psychiatry Sch. Med. Univ. Calif., Davis, CA 95616

To investigate the relationship between lymphocyte β-adrenergic receptor density (BMAX), binding affinity (KD) and psychopathology in depressed outpatients, both were utilized in a multivariate regression model to predict pretreatment scores of the Hamilton Depression Rating Scale (HAM 17), Beck Depression Inventory (BECK), Sheehan Patient Rated Anxiety Scale (SPRAS), State-Trait Anxiety Inventories (X1 and X2) and Chambliss' Accompanied (MOBACC) and Unaccompanied (MOBAL) Mobility Inventories. Forty-six outpatients with major depression, unipolar type (DSM-III-R), (20 females, 26 males) participated. Binding was performed on partially purified lymphocyte membranes using the antagonist ligand 125[I]-iodocyanopindolol (ICYP).

In female patients, BMAX negatively associated with scores on both Mobility Inventories (MOBAL: b'=-0.722; p=0.043; MOBACC: b'=-0.913; p=0.002; Lambda=0.181; p<0.005), but was of no predictive value towards the other measures. In males, neither BMAX nor KD predicted scale scores. These results provide preliminary evidence of an assocation between lowered lymphocyte β-receptor BMAX and severity of panic- agoraphobic symptoms in female depressed outpatients.

BIOLOGICAL RHYTHMS: SLEEP

524.1

INTRACEREBRAL ADMINISTRATION OF ADENOSINE TO THE MEDIAL PREOPTIC AREA ENHANCES SLEEP IN RATS.

S.R. Ticho*, R.M. Virus and M. Radulovacki. Dept. of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612.

To assess the role of adenosine in the medial preoptic area in the sleep-wake cycle of the rat, we have intracerebrally administered adenosine at doses of 5, 25 and 50 nmoles. All doses significantly increased total sleep by 27%, 38% and 28%, respectively, during the 6 hr polygraphic recording period. Analysis of the various sleep states revealed that the enhancement in total sleep was due to a proportional increase in SWS_1 ($p<0.01$), SWS_2 ($p<0.01$) and REM ($p<0.01$) sleep. In addition, doses of 5 and 25 nmoles significantly decreased sleep latencies for all three sleep stages ($p<0.01$). These observations support the hypothesis that adenosine in the medial preoptic area may be involved in the regulation of sleep in rats. Supported by FED-AFOSR contract 85-0349 to MR.

524.2

CHARACTERISTICS OF ADENOSINE A1 RECEPTORS AND ADENOSINE UPTAKE SITES IN THE BRAIN OF NARCOLEPTIC DOGS.

M.Hawkins*, S.O'Connor, E.H.Chen*, S.S.Bowersox, W.C.Dement and M.Radulovacki. Depts. Pharmacology and Epidemiology-Biometry Program, Univ. IL. Coll. Med., Chicago, IL 60612 and Dept. Psychiatry, Stanford Univ. Sch. Med., Stanford, CA 94305.

In order to assess the role of the adenosinergic system in narcolepsy we have examined [^{3}H]R-PIA binding to adenosine (ADO) A_1 receptors and [^{3}H]NBI binding to ADO uptake sites in cerebral cortical membranes isolated from 5 normal and 5 narcoleptic canine brains. Scatchard analysis of [^{3}H]R-PIA binding in sigmoid and coronal gyrus membranes isolated from normal and narcoleptic dogs revealed a single class of high affinity A_1 receptor sites. Our preliminary data also suggest a trend for A_1 receptor upregulation in both cortical gyri of narcoleptic dogs. This is of interest since ADO A_1 receptor upregulation has been found following REM sleep deprivation in the rat brain (Yanik et al., Br. Res., 402:362,1987). Supported by FED-AFOSR-85-0349 to M.R. and NIH NS-19572 to W.C.D.

524.3

LOCALIZATION OF A D SLEEP INDUCTION SITE WITH SHORT LATENCY BY MICROINJECTION OF CARBACHOL IN A HEAD-RESTRAINED CAT.

K. Yamamoto,* A. Mamelak,* J. Quattrochi and J.A. Hobson. Laboratory of Neurophysiology, Harvard Medical School, Boston, MA 02115.

In an attempt to identify a neuroanatomical region which can be chemically stimulated to induce D sleep with very short latency, four cats were placed in a chronic stereotaxic apparatus and microinjected with carbachol (4ug/250nl) using a movable microinjector assembly (Yamamoto et al. 1988). The time from injection until the first D epoch was scored and correlated with injection site. Our results indicate that shortest latencies were found when carbachol was injected in the rostral extent of the anterodorsal pons, a region contiguous with nucleus cuneiformis, central gray, and mesencephalic tegmentum. A map of latency vs. injection coordinate indicates this D induction site is highly localized, since injections greater than 0.5 mm away from this site yield significantly longer (>8 min.) latencies. These results suggest that this region represents a near optimal intersection of multiple neuronal networks involved in D-sleep generation, such that many networks are simultaneously activated by the diffusion of carbachol. Supported by MH 13923.

524.4

EFFECTS OF LY53857, A SELECTIVE 5HT-2 ANTAGONIST ON SLEEP IN THE RAT.

R.H. Pastel. Physiology & Behavior Branch, Dept Medical Neuroscience, WRAIR, Washington, DC 20307.

Recently, we have studied the effects of 3 5HT-2 antagonists (Ritanserin, ICI 169,369 & ICI 170,809) on sleep in the rat (1,2). All three suppressed REM and increased REM latency. Neither ICI compound had an effect on NREM, but Ritanserin disrupted NREM. Others have reported an increase in NREM following ritanserin (3). The present study examines the effects of LY53857, another selective 5HT-2 antagonist. Four male S.D. rats were given saline (ip) and 24 hr later LY53857 (1 mg/kg, ip). Injections were at light onset and sleep was measured for 6 hr after injection. NREM sleep was increased in hr 2-4 after injection and REM was decreased from hr 2-5 following injection. NREM latency was not changed, but REM latency was increased. A decreased number of REM periods was also noted. The present study supports previous findings of REM suppression and increased REM latency following administration of 5HT-2 antagonists. However, the effects of 5HT-2 antagonists on NREM sleep are not consistent. We and others (3) have found an increase in NREM sleep, but in previous studies (1,2) we have found no change in or a disruption of NREM. The role of 5HT-2 receptors in sleep mechanisms is still not resolved.

1. Tortella et al, Brit J Pharmacol (in press). 2. Tortella et al, Brit J Pharmacol (in press). 3. Dugovic & Wauquier, Eur J Pharmacol 137:145, 1987.

524.5

INFECTIOUS CHALLENGE ALTERS SLEEP IN RABBITS. L.A. Toth and J.M. Krueger. Depts. Comparative Medicine and Physiology, Univ. Tennessee, Memphis, TN 38163.

We recently described altered sleep in rabbits after inoculation with Staph. aureus, a gram-positive bacteria (Neurosci. Abstr. 13: 261, 1987). We now report that sleep is also altered in rabbits inoculated with E. coli, a gram-negative bacteria, and Candida albicans, a fungal organism. E. coli increased both slow-wave sleep (SWS) and EEG slow-wave amplitudes (SWA) for the first 4 h postinoculation (PI). SWA were then decreased for the next 20 h. Similar effects occurred if heat-killed E. coli were injected. In contrast, Candida enhanced both SWS and SWA from 6-18 h PI and decreased these parameters from 24-48 h PI. Sleep was not altered after inoculation with heat-killed Candida. Both E. coli and Candida inhibited REM sleep. Sleep responses induced by Candida and Staph. inoculations are similar and may be related to immune stimulation of the host. The more rapid effects produced by E. coli may be related to the presence of heat-stable endotoxin in the cell wall of this organism. (Supported by ONR-N00014-85-K-0773 and NIH-NH25378)

524.6

RATES OF CEREBRAL GLUCOSE UTILIZATION IN THE RAT ARE HIGHER DURING REM THAN DURING SWS. P. Ramm. Dept. of Psychology, Brock University, St. Catharines, Ont. L2S 3A1

Rates of cerebral glucose utilization (CGU) are lower during SWS than during wakefulness (Ramm, P. and Frost, B.J., Brain Research, 365:112, 1986). We have investigated links between CGU and REM. Twenty rats received recording electrodes, and cannulae in the femoral artery and vein. Four days after surgery, the freely-moving animals were REM-deprived for 48 hr. They were then returned to their home cages, where they received intravenous injections of [14C]2-deoxyglucose ([14C]2-DG). During the next 45 min, EEG and EMG recordings were made and arterial blood samples were taken. The animals slept quite normally during the [14C]2-DG incubation period, and many showed elevated levels of REM. Following the incubation period, the brains were extracted, cut, and autoradiographs were prepared. Rates of regional cerebral glucose utilization were calculated by computer densitometry.

Higher rates of CGU were associated with high levels of REM sleep (r_{xy} = 0.64, $p < .05$). Lower rates of CGU were associated with high levels of SWS (r_{xy} = -0.58, $p < .05$). As SWS and REM are differentially linked to fundamental life processes (metabolism), they may also be functionally different.

524.7

VIP-INDUCED REM SLEEP ENHANCES LEARNING IN PCPA PRE-TREATED RATS. O. Prospéro-García*, A. Jiménez-Anguiano*, F. Gil-Holguín*, S. Alvarez-Gallegos*, F. Bermúdez-Rattoni and R. Drucker-Colín. (SPON: J. Velázquez-Moctezuma). Depto. de Neurociencias, Instituto de Fisiología Celular, UNAM. México.

REM sleep has been involved in learning processes. Since it has been shown that VIP has REM sleep-inducing properties in rats and cats, we decided to test the effects of VIP on a learning task (passive avoidance) in parachlorophenylalanine (PCPA) pre-treated rats. In this study, we used male Wistar rats (180-210 g). They were implanted for standard sleep-wake cycle recordings. Additionally, a stainless steel cannula (22 gauge) was implanted into the IV ventricle. Rats were treated with 2 i.p. injections of PCPA, 24 h. apart. Twenty-four hours after the 2nd injection of PCPA, rats were placed in a step-through apparatus to train them. Immediately following acquisition, the rats received an intraventricular injection of either saline (5 ul) or VIP (200 ng). After this treatment, rats were recorded for 24 hrs. Upon completion of the recording period, a retention test was performed. It was found that VIP significantly increased total time of REM sleep as compared to saline (54.82 $\pm$ 8.78 min vs 5.55 $\pm$ 1.46 min). Whereas passive avoidance tests showed that VIP group had a prolonged step-through latency with respect to saline group (342.5 $\pm$ 96.5 sec vs 28.5 $\pm$ 16.5 sec). These results strongly suggest that VIP-induced REM sleep enhances learning in rats.

524.8

MEDIAL RETICULAR FORMATION (MRF) UNIT ACTIVITY DURING AUDITORY STIMULATED REM SLEEP PERIODS. G. Arankowsky*, D.J. McGinty and R. Drucker-Colín. (SPON: Graciela Meza). Depto. de Neurociencias, Instituto de Fisiología Celular, UNAM, México, D.F.

It has been reported that auditory and somatic stimulation during rapid eye movement (REM) sleep is capable of increase REM sleep duration. The present work attempts to determine whether changes in MRF unit activity are related with this phenomenon. Two cats were implanted under pentobarbital anesthesia with electrodes for conventional polysomnographic recording. Also, two barrel microdrives each housing six microwires were targeted into the MRF. The sleep-waking cycle was recorded alternating control REM sleep periods with auditory stimulated ones (2 KHz, 90 dB, every 20 sec) and the units discharge rate was determined. 17 Cells were recorded; while 7 units (41%) were not responsive to stimulation; 10(58%) were responsive to the stimulus. 7 Of these did not show changes in their discharge rate when comparing control vs stimulated periods. Three cells increased the mean discharge rate during stimulated periods ($p < 0.05$):

Control (Sp./9 sec)	Stimulus (Sp./9 sec)
5.12 $\pm$ 1.5	8.51 $\pm$ 2.00
32.2 $\pm$ 15.6	39.3 $\pm$ 12.05
5.5 $\pm$ 2.0	9.0 $\pm$ 4.0

Our results suggest that REM sleep enhancement due to sensory stimulation could be mediated by an increase in the excitability levels of the MRF.

524.9

BIPHASIC ACTION OF IBOTENIC ACID INJECTION INTO MEDIALIS DORSALIS THALAMIC NUCLEUS ON SLEEP BEHAVIOR AND EEG PATTERNS IN CATS. G. Marini*, I. Gritti and M. Mancia (SPON: European Brain and Behavior Society) Ist. Fisiologia Umana II & *ITBA via Mangiagalli 32-Milano-Italy

The medialis dorsalis thalamic nucleus (MD) has recently been suggested to subserve a role in inducing and maintaining sleep on the basis of both clinical and experimental studies. In order to further investigate its role in the control of brain waves and behavioral sleep, very localized chemical lesions were placed into MD by means of cell-selective neurotoxin ibotenic acid (IBO) injections. Five adult cats were implanted for chronic sleep recordings, under general anesthesia, according to standard techniques. One week later, under ketalar anesthesia, a Hamilton microsyringe needle was guided stereotaxically into the intermediate-lateral portion of MD and IBO (50µg/µl, 1-2µl) was slowly injected. Polygraphic recordings were made in pre- and post-lesioned unrestrained animals. The location of the lesion was histologically confirmed. Immediately after the IBO microinjections a behavioral hypersomnia lasting 8-28 hrs was observed. Quantitative spectral analysis done in 2 animals during this state revealed a sudden significant ($p<0.05$) increase in the 6-12 Hz activity followed by a gradual reduction up to a transient suppression. After unilateral IBO MD-injections in one animal, a remarkably long-lasting asymmetry was observed in the spindle activity (reduced in the lesioned side). Subsequently, the spindle activity recovered and EEG desynchronization and hyposomnia (residual sleep was light and fragmented) gradually developed in all animals. These results fit in nicely with the hypothesis that subsets of MD neurones play a role in synchronizing electrocortical activity and support the excitotoxic hypothesis of glutamate analogues.

524.10

QUISQUALIC ACID LESIONS OF THE VENTROMEDIAL MEDULLARY RETICULAR FORMATION: EFFECTS UPON SLEEP-WAKEFULNESS STATES. C.J. Holmes, H.H. Webster*, S. Zikman* and B.E. Jones. Department of Neurology & Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

Since the classical neurophysiological studies of Magoun and Rhines, it has been assumed that the motor inhibition occurring during paradoxical sleep (PS) depends upon neurons in the ventromedial medullary reticular formation (VMRF) which project to the spinal cord. To test this assumption, we have selectively destroyed neurons in this area with stereotaxic injections of quisqualic acid (90ug) in cats implanted with standard electrodes for EEG, EMG, PGO and EOG. The animals were recorded 24 hours per day one week prior to and one month subsequent to the lesion.

The daily time spent in waking, slow wave sleep and PS was not greatly altered, but neck muscle tonus was significantly increased in PS during the full month of recording following the lesions. This increased tone was associated with whole body movements, including vigorous movements of the limbs. Histological analysis of the tissue revealed extensive cell loss in the VMRF. These results support the contention that the cells of the ventromedial medullary reticular formation contribute to muscle atonia during paradoxical sleep in the cat. (Supported by MRC of Canada)

524.11

CHOLINERGIC NEURONS FROM THE DORSAL PONS PROJECT TO THE MEDIAL PONS. P.J. Shiromani, D.M. Armstrong, and J.C.Gillin. San Diego VAMC & Univ California, San Diego Dept Psychiatry & Neuroscience (DMA), La Jolla, CA,92161

Infusions of cholinergic agonists into the medial pons readily triggers REM sleep. However, since there are no cholinergic cells in the medial pons, a cholinergic input is required to depolarize the medial pontine neurons important for physiologic REM generation. We examined the cholinergic input to the medial pontine area.

Four cats were deeply anesthetized and stereotaxic microinjections of WGA-HRP (5%;0.1ul-0.05ul) were made into the medial pons. 48 hrs later the cats were administered Nembutal and perfused. 40 um thick sections were processed for HRP using TMB as the chromagen. The same sections were then placed in a polyclonal antibody to ChAT (provided by L. Hersh) which determined whether the HRP labelled neuron was cholinergic.

We observed numerous HRP- and ChAT-labelled cells scattered throughout the peribrachial region and lateral dorsal tegmental (LDT) region. Estimates based on gross examination of the area suggest that less than 20% of the cholinergic cells in the LDT and peribrachial region were double-labelled.

These results demonstrate a cholinergic input from the pedunculopontine region and we suggest that REM sleep occurs when acetylcholine from these neurons depolarizes the medial pontine cholinoceptive neurons.

524.12

EFFECTS OF TONE INTENSITY ON PGO WAVES. W.A.Ball*, W.K. Hunt*, R.J. Ross, and A.R. Morrison. Lab. of Anatomy, Sch. of Vet. Med., U. of Pa., Phila., PA 19104.

Tones elicit PGO waves in both slow wave sleep (SWS) and paradoxical sleep (PS); rapid habituation of the effects occurs in SWS, but less change is observed in PS (Ball, W.A. et al, Sleep Res., 17:1988). In the early trials, however, tones elicit PGO waves more readily during SWS than PS. We tested the effects of tone intensity on this initial difference.

Six cats were implanted with standard lateral geniculate, electroencephalogram, electrooculogram, and electromyogram electrodes. Tones (1000 Hz, 90 msec square wave) were presented as blocks of 5 different intensities (60, 70, 80, 90, and 100 dB), each block tested in up to 8 sessions in each state. FM recordings were made for off-line computer analysis.

In SWS tones elicited PGO waves more often than in PS ($p<.01$) at all intensities and tended to have shorter mean latencies to peak ($p<.04$, 83 vs.101 msec.). From 60 to 100 db, PGO waves elicited in PS and SWS became 1) more frequent ($p<.01$) 2) 17 and 42 % greater in amplitude in PS and SWS respectively ($p<.01$) and 3) reduced in latency (96 and 85 msec at 60 and 100 db, $p<.05$).

Thus, external stimuli are less likely to elicit PGO waves during PS. We speculate there may be sensory gating or ceiling effects in the activity of PGO generating sites. Supported by 1-RO1-MH42903-01 and F32-MH-09584-01.

524.13

SUPPRESSION OF MUSCLE TONE PRODUCED BY ELECTRICAL STIMULATION AT THE PONTO-MESENCEPHALIC JUNCTION. Y. Y. Lai and J. M. Siegel. Sepulveda VAMC, UCLA School of Medicine, Sepulveda CA 91343

Stimulation of the medial medulla and dorsolateral pons produce a suppression of muscle tone. These regions have been implicated in the loss of muscle tone in REM sleep and in cataplexy. In the present study, we report that stimulation of the dorsolateral portion of the reticular formation at the ponto-mesencephalic junction also produces bilateral inhibition of muscle tone. Studies were performed on 4 unanesthetized decerebrate cats. Trains of cathodal pulses (100 Hz, 0.2 ms, 10-100 μA) were delivered through stainless steel monopolar microelectrodes to the area between A2.0-P1.0, L 0-5.5, H+2.0 to H-9. Bilateral recordings were made from neck muscles (occipitoscapularis, splenius, and biventer cervicis). We found that stimulation between A2.0-A0.0, L3.0-5.0, H0 to -2.5 produced bilateral inhibition of muscle tone at very low stimulation intensity thresholds (15-50μA). This area corresponds to the lateral portion of the cholinergic pedunculopontine tegmental nucleus, and is just rostral to the pontine cholinoceptive region known to trigger atonia. Cell bodies or axons in this area may participate in generating the atonia of REM sleep and cataplexy, or may help regulate postural tone in waking.

524.14

RECEPTORS MEDIATING SUPPRESSION OF MUSCLE TONE PRODUCED BY GLUTAMATE IN DORSOLATERAL PONS AND MEDIAL MEDULLA. J. M. Siegel and Y. Y. Lai. Sepulveda VAMC, UCLA School of Medicine, Sepulveda CA 91343

We have previously reported that suppression of muscle tone can be produced from three brainstem sites implicated in REM sleep atonia. Acetylcholine [Ach] microinjected into the dorsolateral pons [DLP], or nucleus paramedianus of the medulla [NPM], and glutamate in DLP or nucleus magnocellularis of the medulla [NMC] produce atonia in the decerebrate cat. Gamma-D-glutamylglycine [DGG], an NMDA and kainate receptor antagonist, blocked the glutamate-induced muscle inhibition in DLP and NMC. L-glutamic acid diethyl ester [GDEE], an NMDA and quisqualate receptor antagonist, blocked or attenuated the glutamate effect on muscle activity in both DLP and NMC. However, DL-2-amino-5-phosphonovaleric acid [APV], a highly specific NMDA receptor blocker, did not block the glutamate-induced atonia in DLP or NMC. Both Ach and its agonist, carbachol, induced muscle atonia in DLP and NPM but not in NMC. Microinjected atropine blocked the Ach effect on muscle activity at both sites. Agonist studies confirmed the antagonist findings. NMDA injection into either DLP or NMC produced excitatory effects on muscle activity. In contrast, kainic and quisqualic acid induced bilateral inhibition. We conclude that non-NMDA and muscarinic receptors mediate DLP atonia. Non-NMDA receptors mediate NMC atonia. Muscarinic receptors mediate NPM atonia.

524.15

CHANGE IN PONTINE UNIT RESPONSE TO AUDITORY STIMULI AFTER REM SLEEP DEPRIVATION. B. N. Mallick*, H. Fahringer, M. F. Wu and J. M. Siegel (SPON: R. Szymusiak). Sepulveda VAMC, Sepulveda CA 91343, UCLA Sch. Med., Los Angeles CA

While the behavioral changes resulting from REM sleep deprivation have been extensively analyzed, the neuronal substrates of these changes are unknown. In the present study, we have taken advantage of the extraordinary stability of the microwire recording technique to analyze changes in unit activity during REM deprivation. We have studied 8 cells in the dorso-lateral pontine reticular formation, an area critical for REM sleep control. Units were recorded continuously for at least 40 hours during baseline, deprivation and recovery periods. Post-stimulus histograms of response to 102-105 db tones, were computed. During predeprivation periods, stimuli produced an inhibition of discharge with a latency of 20-40 msec. REM deprivation produced a reduction in the evoked inhibition ($p<0.002$, F test). Responses returned to baseline levels after 20 hours of recovery sleep. Discharge rate did not change during deprivation. Clonidine administration produced a similar reduction in evoked auditory inhibition. Recent studies have shown that REM sleep deprivation produces downregulation of norepinephrine receptors. This downregulation/desensitization is hypothesized to mediate the present effect and may underlie many of the behavioral and physiological effects of REM deprivation.

524.16

LATERAL GENICULATE SPIKES, MUSCLE ATONIA AND STARTLE RESPONSE ELICITED BY AUDITORY STIMULI AS A FUNCTION OF STIMULUS PARAMETERS AND AROUSAL STATE. M.-F. Wu, B. N. Mallick* and J. M. Siegel. Neurobiology Res., VAMC, Sepulveda, CA 91343 and UCLA Med. Ctr, Los Angeles, CA 90024.

The occurrence of ponto-geniculo-occipital (PGO) waves has been associated with phasic suppression of both muscle tone during non-REM sleep and monosynaptic reflexes during REM sleep. However, muscle excitation and reflex enhancement in association with PGO waves have also been noted. In the present study, we have systematically examined the effect of auditory stimuli on PGO and EMG as a function of stimulus intensity and arousal state. Clicks from 65 to 115 dB were presented. EMG responses were averaged and peak-to-peak LGN PGO amplitudes measured. We found that stimuli >95 dB produce temporally separated EMG suppression and excitation, with the excitatory response maximal in waking, while the inhibitory response was maximal in the pre-REM period. <75 dB stimuli mainly produced muscle suppression. PGO waves could be elicited with both low and high intensity stimuli, with the amplitude proportional to intensity. PGOs evoked by equal intensity stimuli were of progressively larger amplitudes in waking, non-REM, REM and pre-REM. These findings demonstrate that the amplitude of PGO waves and the direction and amplitude of EMG responses to auditory stimuli, are a function of both stimulus intensity and behavioral state.

524.17

EFFECT OF REM SLEEP DEPRIVATION IN THE DEPTH PERCEPTION ON THE VISUAL CLIFF IN RATS OF 30 AND 60 DAYS. Cintra, L., Díaz-Cintra, S., Labastida, P*., Galván, A*. and Ortega A*. Dept. of Physiology Inst. Inv. Biomédicas, UNAM, México, City, C.P. 04510.

This study was performed to know the effect that REM sleep deprivation (RSD) by the platform method, produces in the depth perception on the visual cliff apparatus in rats of 30 and 60 days. For the visual cliff procedure, rats were REM sleep deprived by 48h and at the same time two trials of 3 min each were performed at 12h intervals; as well as in the recovery day. Vigilance states of animals implanted in the occipital cortex and neck muscles, were EEG recorded during 24h of control day and during the recovery day after 48h of RSD. No significant differences were found at 30 days between sleep deprived (SD) rats and control (C) rats however, the SD rats at 60 days showed preferences for the deep side (DS). When we compared each group by age, was clear that SD rats of 30 days preferred the shallow side (SS) and 60 days animals the DS; C rats showed no differences. REM sleep presented a significant increment on both ages during the recovery day in the 12h rest phase and slow wave sleep, increased significantly during activity phase at 30 days, and REM sleep at 60 days; 24h analysis revealed a significant decrease in waking in both ages and significant increase in REM at 60 days. These results agree with the hypothesis that 48h of RSD, affects during this period the visual depth perception in rats, due to alterations in the visual and motor systems.

524.18

RESPIRATORY DISTURBANCE INDEX CHANGES WITH AN ANTERIOR MANDIBULAR POSITIONING DEVICE FOR OBSTRUCTIVE SLEEP APNEA. G.T. Clark, D. Arand* and E. Chung* (SPON: M.C. Carter). UCLA Dental Research Inst. & Sleep Disorders Clinic, Neuropsychiatric Inst., Los Angeles, CA 90024.

Several case reports have indicated that dental devices have a potential effect in the reduction of obstructive sleep apnea (OSA) problems. This study utilized an anterior mandibular positioning appliance in OSA patients and evaluated the changes in their respiratory disturbance index (RDI). The RDI is calculated by dividing the total sleep time into the total number of oxygen desaturations. The amount of movement induced by the appliance was 60-80% of the mandible's maximum protrusive distance (5-7mm) measured from maximum intercuspation. The RDI levels were determined during all night polysomnographic studies. To date, 5 diagnosed OSA subjects have completed all phases of the protocol which involved a pre-appliance sleep study, a 1-month post-appliance sleep study, and a 6-month post-appliance clinical examination. All 5 subjects showed a clear decrease and the mean and s.d. for the RDI level pre-appliance was 28.3 ± 7.3 and 4.24 ± 1.93 post-appliance. The 6-month clinical examination data clearly indicates no adverse change in jaw function (range of motion, joint sounds or muscle tenderness) and patients continue to be fully compliant with the prescribed all night use of the appliance.

524.19

SLEEP-ONSET CRITERIA IN THE DETERMINATION OF EXCESSIVE DAYTIME SOMNOLENCE. C. P. Browman. Sleep Disorders Center, Humana Hospital-Audubon, Louisville, KY 40217.

Sleep onset on the multiple sleep latency test (MSLT) was determined using a single epoch of stage 1, three consecutive epochs of stage 1, and a single epoch of stage 2 or REM sleep for 21 patients (mean age 38.0 yr) with narcolepsy and 21 patients (mean age 38.0 yr) with obstructive sleep apnea (mean apnea index of 64.3). Five MSLT procedures were from 10:00 to 18:00. The resulting polysomnogram was scored in 30 sec epochs. For patients with narcolepsy, mean sleep latency in min using one epoch of stage 1 was 3.07±0.35, using three epochs of stage 1 was 3.37±0.37, and one epoch of stage 2 or REM was 5.97±0.55 (p<.001). The respective values for patients with sleep apnea were 3.61±0.54, 4.36±0.61, and 8.46±0.85 (p<.001). Measures of sleep latency were interrelated for patients with narcolepsy (r=.969 to .701, p<.001) and for patients with sleep apnea (r=.894 to .751, p<.001). The obstructive apnea prevented or delayed sleep onset 18.14% of trials using one epoch of stage 1, 24.76% using three epochs of stage 1, and 27.62% using one epoch of stage 2 or REM criteria for patients with sleep apnea. A single epoch of any stage of sleep is an appropriate measure of sleep latency for narcoleptics although a modified scoring system should be developed for sleep apneics and other patients to account for the pathology-related interruptions.

524.20

EEG: CHAOS AND QUASIPERIODICITY. H. Stowell. ERBP Lab., 120 Nature Creek, Milledgeville GA 31061.

The mass-action of cooperating/competing neural networks interests artificial-intelligence (AI) and neurocomputing engineers (Denker, J.S., AIP Conf. Proc. 151. AIP, New York 1986). The mass-action of one mammalian sensory nucleus, the olfactory bulb, now approaches intelligibility in terms of nonlinear dynamics (Freeman, W.J. et al., Behav. Neurosci., 100: 753; 101: 393, 766, 1986-7). Is it useful to examine human EEG in similar terms (Stowell, H. Int. J. Neurosci., 32: 861; 34: 117; 35: 111, 1987)? Though it remains impossible to discard the hypothesis that extracellular slow waves of the brain reflect noise irrelevant to information processing, some progress appears in frequency analysis of time-domain event related brain potentials (ERBP), showing that: (i) Synchronization of "averaged evoked EP" reflects a transient perturbation of ongoing EEG, constraining its possible phases (Sayers, B.McA. et al., Nature, 274: 481, 1974); (ii) Similar phase order occurs in the 300 ms before a familiar stimulus and, under specifiable conditions of subjective time-frame, at the expected times of absent pattern elements during subjective discrimination of acoustic rhythms. Progress in testing the hypothesis of a functional EEG will need powerful algorithms with massive data processing (Gevins, A.S. et al., Science, 235: 580, 1987) and support from AI (Cohen, M.S. & Julian, W.H. Neural Networks with a Hopf Bifurcation: Slowly Modulated Waves. Personal communic. from CRL, Las Cruces NM, 1987).

524.21

EEG FACTOR SCANS. R. Gussio*, K.L. Marquis*, G.G. Buterbaugh and G.A. Young. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Attempts to quantify EEG are typically accomplished by period amplitude analysis, Fourier analysis, etc. Data from these analytical techniques are usually subjected to univariate statistical evaluations which are limited by their inability to statistically control multidimensional data sets. Therefore, we combined the powerful features of single case design, derived power spectral parameters, factor analysis and computerized three dimensional graphics in the development of EEG factor scans. Frontal-parietal cortical EEG recordings were collected during sleep-awake cycles, drug-induced states and status epilepticus in rats. Quantitatively and qualitatively unique EEG factor scan patterns were generated from each of these CNS states. As an example, the relatively selective mu and kappa agonists morphine and ethylketocyclazocine were distinquished by the patterned loadings of several factors which are independent from the relative distribution of power spectral amplitudes within descrete frequency bands. These results illustrate the sensitivity of this novel assay. We predict that EEG factor scans will have utility in the classification of disparate groups of CNS active agents and pathological states. (Supported in part by USPHS grants NS20670 and NIDA DA01050)

525.1

AGGREGATE CULTURES OF RAT FETAL DOPAMINE (DA) NEURONS PRODUCE BEHAVIORAL EFFECTS IN PARKINSONIAN RATS. J.F. Loring, R.E. Strecker, B. Boss, R. Miao*. Hana Biologics, 850 Marina Village Parkway, Alameda, CA 94501

Neural grafts of freshly dissected DA-rich fetal ventral mesencephalon (VM) restore function in rats made parkinsonian by 6-OHDA treatment. If graft therapy for Parkinson's disease is to be successful, it may become important to maintain the tissue in culture before grafting. However, it is likely that neurons that grow processes onto planar substrata suffer damage during dissociation required to remove them from culture dishes for grafting. To avoid this problem, we have used aggregate tissue culture methods, which allow cultured DA-rich neuronal tissue to be grafted after only one initial dissociation procedure. Donor tissue was dissected from the VM of 13 day old rat fetuses and prepared for aggregate culturing by a modification of the method of Hemmendinger et al. (PNAS 78:1264-1268; 1981). Cells were cultured 9 days in rotating flasks. The cells formed many small balls (175-300 um dia.) each estimated to contain over 5,000 cells. Forty such balls were injected via a 22 G needle into the DA-denervated striata of host parkinsonian rats. Six weeks post grafting 7 of 11 rats exhibited behavioral signs of a functional graft, as shown by both reduction in amphetamine-induced ipsilateral rotation and increase in contralateral rotations. One rat sacrificed at 3 weeks post grafting had over 400 surviving dopaminergic neurons, judged by tyrosine hydroxylase (TH) immunohistochemistry. TH-positive fibers extended from the transplant site into the host striatum. These results suggest that aggregate culture methods are a promising means to maintain and deliver fetal neurons for graft therapy.

525.2

ENCAPSULATED EMBRYONIC MOUSE MESENCEPHALIC TRANSPLANTS ALLEVIATE EXPERIMENTAL PARKINSONISM IN RATS. P.Aebischer*, S.R. Winn*, D. Ross (SPON: J. Parmelee). Artificial Organ Laboratory, Brown University, Providence, RI 02912.

Embryonic mesencephalic xenotransplants alleviate experimental parkinsonism, however they are rejected over time. We are studying the ability of cografts of mesencephalon and striatum immunoprotected by a permselective membrane to affect experimental parkinsonism. A polymeric membrane with a molecular weight cut-off of 50,000 daltons provides immunoprotection by allowing nutrients, growth factors and neurotransmitters to freely diffuse through the membrane while preventing the invasion of immunoglobulins and cells. Polyvinyl chloride acrylic copolymer permselective tubes, ID 600 µm, were loaded with E14 mouse cografts and capped with a compatible polymer glue. The polymer capsules were then placed stereotaxically in the striatum of 6-hydroxydopamine lesioned rats. Rats implanted with empty polymer capsules served as controls. Motor asymmetry was assessed under amphetamine (5 mg/kg) stress.Cohorts of 6 animals were observed for at least 8 weeks. A significant decrease in rotational behavior was observed in 5 of the 6 encapsulated cografts whereas no decrease was observed with empty polymer capsules. Upon retrieval intact neuron-like cells were observed within the polymer capsules up to 3 months of implantation. Tissue reaction to the polymer capsule was minimal. These results indicate that synaptic contact is not required in order to reduce the symptomatology of experimental parkinsonism. Whether the effects observed are due to dopamine release or host regeneration induced by growth factors released from the transplants is not known.

525.3

INTRASTRIATAL IMPLANTATION OF AN ENCAPSULATED L-DOPA RELEASING POLYMER REVERSES EXPERIMENTAL PARKINSONISM IN RATS. S.R. Winn *, A.N. Salessiotis *, P.Aebischer * (SPON: N. Knuckey). Artificial Organ Laboratory, Brown University, Providence RI 02912.

Parkinson's disease is characterized by a deficit of dopamine within the striatum. We are investigating the feasibility of implanting a polymeric substrate which releases L-DOPA to reverse experimental parkinsonism. Films of poly[ethylene vinyl acetate] containing 10% by weight of L-DOPA were cast, shaped into cylinders, and encapsulated within permselective acrylic copolymer tubes with a nominal molecular weight cut-off of 50,000 daltons. The tubes were sealed with a compatible polymer glue and implanted stereotaxically in the striatum of 6-hydroxydopamine lesioned rats. Cohorts of 3 animals received L-DOPA loaded capsules; controls received capsules loaded with substrate alone. Rotational behavior was observed under amphetamine challenge (5 mg/kg). Immediately following transplantation, animals with L-DOPA loaded capsules spontaneously rotated contralateral to the lesioned side. At 2 to 4 days post-transplantation, net rotational asymmetry under amphetamine stress was reduced 100% to that of pre-transplant rotational values. One week post-transplantation, rotational behavior in animals with the L-DOPA containing capsules returned to pre-transplant values. No changes in post-transplantation rotational asymmetry were observed in control animals. The present study shows that intrastriatal release of L-DOPA suspended in a hydrophobic polymer is able to reverse experimental parkinsonism in rats. Controlled release of neurotransmitters or macromolecules from a polymeric substrate may provide an alternative method of treatment for neurological disorders

525.4

XENOGRAFTS OF FETAL PIG DOPAMINE (DA) NEURONS SURVIVE AND FUNCTION IN BOTH CYCLOSPORIN A (CyA)-IMMUNOSUPPRESSED AND IMMUNODEFICIENT NUDE RATS. R.E. Strecker, T. Huffaker*, B.Boss, J.F. Loring, A. Morgan*, M. Spence*, A. Xuan*, and R. Miao*. Hana Biologics, 850 Marina Village Parkway, Alameda, CA 94501.

Fetal pig CNS is an attractive model system to use for the development of a graft therapy for Parkinson's disease since, relative to rats, the developmental time course of the pig more closely approximates that of the human embryo, and pig DA neurons can be expected to innervate a larger brain area than rat neurons. Longterm survival of CNS xenografts in rats has been shown to require immunosuppression with CyA, a time-consuming and expensive procedure. Hence, we tested whether fetal pig CNS grafts would survive in both nude rats and immunosuppressed normal rats. DA-rich cell suspensions of freshly dissected embryonic day 21 fetal pig ventral mesencephalon were grafted into the right striatum of 20 CyA-immunosuppressed and 8 nude rats. All rats had previously been made unilaterally parkinsonian with 6-OHDA. At 9 weeks post-grafting 15 of 20 CyA-treated rats exhibited behavioral effects of the grafting, as shown by a reduction of amphetamine-induced rotation, with complete reversal of rotation by 14-17 weeks. Cessation of CyA treatment resulted in behavioral signs of graft rejection within 4 to 9 weeks. Control grafted rats (N=9) showed no change in rotation. Large numbers of DA neurons (identified by tyrosine hydroxylase immunohistochemisry) were found in functional grafts. Grafted nude rats showed a similar reduction in amphetamine-induced rotation, which remained stable for at least 18 weeks, indicating that the nude rat model is a useful alternative to CyA injections in CNS-xenografting experiments.

525.5

DISPLACEMENT OF MPP^+ FROM ITS BINDING SITES AND INHIBITION OF MAO-A ACTIVITY BY DEBRISOQUIN AND SOME ANALOGUES IN MOUSE BRAIN. M.Del Zompo, S.Ruju*, R.Maggio*, M.P.Piccardi*, G.U.Corsini*. Dept. Neurosciences, Clin. Pharmacol., Univ. of Cagliari, Italy.

Self-administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes irreversible parkinsonian symptoms in man. It has been proposed that MPTP is effective by conversion via oxidation to an active metabolite, 1-methyl-4-phenyl-piridine (MPP^+). Several findings suggest that type B monoamine-oxidase (MAO-B) is responsible for the oxidation of MPTP to MPP^+. We have previously shown that ^3H-MPP^+ binds saturably and with high affinity to mouse brain membranes. Successively, an adrenergic neuron blocking agent, debrisoquin, was found to compete with ^3H-MPP^+ for its binding sites with a IC50 of 15± 2 uM. In order to better characterize this property, several analogues of debrisoquin have been tested. The relative structure required to inhibit MPP^+ binding sites has been evaluated.The derivative with a tiocarbamydic group at the position R4 is essential for the affinity for the ^3H-MPP^+ binding sites. Moreover these analogues have been tested to determine MAO inhibiting activity, being debrisoquin a potent intraneuronal MAO-A inhibitor. A correlation between these structure requirements for binding sites and MAO inhibition capacity has been evaluated.

525.6

EFFECTS OF MPTP ON THE FINE STRUCTURE OF NIGRAL NEURONS OF DOG: POSSIBLE EVIDENCE OF CALCIUM LOADING. V.O.F. Warrington*, S.C. Rapisardi and J.S. Wilson, Dept. Anatomy, Howard Univ., Washington, D.C., 20059.

For the last several years, we have been systematically studying the spatial and temporal evolution of degeneration induced by MPTP in the nigrostriatal system of dog. This is our first report of fine structural changes. Two dogs received a single injection of MPTP (3mg/kg; i.v.) and were sacrificed 1 and 4 days later. A third dog received an injection of saline and served as a control. The brains were stereotaxically blocked into 5 mm slabs, and cores of tissue were taken with a 18 guage needle from the medial pars compacta of the substantia nigra. The locations of cores were verified histologically using immunostaining for tyrosine hydroxylase. Cores were processed for conventional electron microscopy.

In the MPTP treated animals, major changes were seen in the rough endoplasmic reticulum (RER) and mitochondria. In single sections from the normal animal, the RER appeared as small rectangular stacks which occupied approximately 5.0% of the total area of the soma. The longest continuous membrane with bound ribosomes in each array averaged 0.0014 mm. In the one day survival animal, the RER appeared in very long, uninterrupted ribbon-like arrays (longest average length/array=0.014mm) which occupied approximately 18.3% of the total area of the soma. In the four day survival animal, the RER was disrupted and only small fragments of membrane bound ribosomes were observed.

Mitochondria demonstrated a tremendous swelling in the MPTP treated animals changing their average area from 8.2xE-8 in the normal animal to 1.6xE-7 and 6.2xE-7 in the 1 and 4 day survival animals, respectively. Although the mitochondrial matrix remained electron lucent, their cisternae were completely disrupted in the longer survival animal.

Similar mitochondrial changes have been reported in other pathological models in which the neurotoxic effects are thought to be the consequence of calcium overload such as kainic acid poisoning, cerebral ischemia and seizure. We have recently reported that incubation of mouse brain slices in calcium-free medium blocked MPTP's toxic effects (Wilson, Neurosci. Abst., 12:1309, 1986). These results provide further support that intracellular calcium accumulation may play an important role in MPTP's toxicity. NIH-MBRS 2S06-RR-08016-16.

525.7

MPTP PRODUCES A MOSAIC PATTERN OF TERMINAL DEGENERATION IN DOG:FURTHER EVIDENCE FOR SUBCOMPONENTS OF THE NIGROSTRIATAL SYSTEM.

B.H. Turner, J.S. Wilson and J.C. McKenzie*, Dept. Anat., Howard Univ., Washington, D.C. 20059.

We have previously reported that MPTP, a toxin specific to the dopaminergic nigrostriatal pathway, produced a mosaic-like pattern of terminal degeneration in the caudate nucleus (Cd) of dog (Brain Res. 423:329-332, 1987). The purpose of this study was to determine if this mosaic-like pattern corresponded to the striosome/matrix compartments as defined by acetylcholinesterase (AChE) staining. We found that in the normal dog both AChE and tyrosine hydroxylase (TH) histochemistry defined a matching mosaic organization of the Cd nucleus that was similar to that described in other species. A single injection of MPTP (3.0 mg/kg; i.v.) produced a mosaic pattern of terminal degeneration in the Cd nucleus which was most pronounced at 8 to 10 days survival. Comparing sections stained for terminal degeneration by the Fink-Heimer method with adjacent sections stained for AChE and TH revealed that heavy degeneration was limited to the matrix while only light degeneration was found in the striosome. At no survival times examined thus far (1-14 days) has heavy terminal degeneration been observed in the striosome compartment. This may be because the dopaminergic projection to the striosome 1) may have a higher toxic threshold to MPTP, 2) may be less dense or 3) may be more difficult to stain with silver degeneration methods. These data provide further evidence that the nigrostriatal system is composed of parallel projections to the striosome and matrix. Supported in part by NIH-MBRS Grant 2S06 RR 08016-16.

525.8

MPTP CAUSES AN ACUTE DECREASE IN DOPAMINE (DA) LEVELS IN MOUSE BRAIN SLICES. J.A. Wilson, T.J. Doyle*, J.G. Gleason*, & Y.S. Lau. Depts. of Physiology & Pharmacology, Creighton Univ. School of Medicine, Omaha, NE 68178.

MPTP causes a Parkinson's disease like decrease in DA levels in man and certain other animals. It can selectively kill neurons originating in the substantia nigra, but in some animals, recovery occurs indicating that the lesions were not permanent. The cause of this acute decrease in DA content by MPTP is not well known. Both MAO inhibitors (pargyline) and DA uptake inhibitors (GBR-12909) can prevent the toxic effects. We investigated the effects of MPTP on DA content in mouse brain slices. Comparison of control brain slices with MPTP treated brain slices shows that a 20 min. application of 300 μM MPTP causes a significant reduction in DA content. Treatment with either pargyline, GBR-12909, or low Ca^{++}-high Mg^{++} alone causes no change in DA content; however, co-application of these compounds with MPTP results in DA levels significantly lower than control levels and not different from the DA levels of slices treated only with MPTP. The MPTP induced decrease in DA content could be prevented by γ-hydroxybutyrate (a DA release inhibitor). We interpret these results to indicate that MPTP induced depletion of DA *in vitro* is not causally related to MPTP's ability to selectively lesion DA neurons.

Supported by a grant from the Health Future Foundation.

525.9

THE NEUROPATHOLOGY OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDRO PYRIDINE: HOW IMPORTANT IS MITOCHONDRIAL DAMAGE?

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1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin which destroys dopaminergic neurons in the mouse substantia nigra. In vitro evidence indicates that MPTP is a mitochondrial toxin which inhibits the electron transport chain. However, there is also a possibility that MPTP acts through the generation of oxygen radicals which are toxic to mitochondria as well as most other cell organelles. This study found that the primary lesion produced by MPTP was damage to astrocytes. Both the protoplasmic and fibrous astrocytes were affected. At the subcellular level, endoplasmic reticula were more affected than mitochondria at early time points. This study has demonstrated that MPTP is not exclusively a mitochondrial toxin. All animals were cared for in accordance with NIH guidelines. At no time during this study did animals suffer. This research was supported by NIH grant NS23515.

525.10

EFFECTS OF SELECTIVE D_1 AND D_2 RECEPTOR BLOCKADE ON LEVODOPA-INDUCED REGIONAL GLUCOSE UTILIZATION CHANGES.

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Following systemic L-dopa administration to rats with unilateral 6-hydroxydopamine substantia nigra lesions, regional cerebral glucose utilization (RCGU) increases 2-3 fold in the ipsilateral entopeduncular nucleus (EP) and substantia nigra pars reticulata (SN_r) and decreases in the ipsilateral lateral habenula (LHN) (Brain Res. 379:264). In this study we have examined the effects of selective D_1 and D_2 receptor blockade on dopa-induced RCGU changes. Lesioned rats were administered haloperidol 3 mg/kg i.v. or SCH 23390 1 mg/kg i.v. (to block D_2 or D_1 receptors, Brain Res. 415:90), followed 1 hour later by L-dopa methyl ester 25 mg/kg i.v. and [^{14}C]2-deoxyglucose 10 μCi/100 g i.v. SCH 23390 nearly completely blocked (90%) the dopa-induced RCGU increase in the EP and SN_r. Haloperidol also effectively attenuated the RCGU increase by 70%. Neither drug blocked the effects of dopa on RCGU in the LHN. Both haloperidol and SCH 23390 markedly attenuated dopa-induced rotational behavior. We conclude that dopamine formed following decarboxylation of L-dopa stimulates both D_1 and D_2 receptors in vivo. While D_1 stimulation appears necessary, both D_1 and D_2 receptor occupancy contribute to the marked RCGU increase in EP and SN_r following L-dopa.

525.11

L-DOPA-INDUCED ROTATIONAL BEHAVIOUR IS DEPENDENT ON BOTH STRIATAL AND NIGRAL MECHANISMS.

G.S. Robertson and H.A. Robertson* Dept. of Pharmacology, Dalhousie University, Halifax, N.S. Canada B3H 4H7

Recent evidence suggests that the therapeutic action of L-Dopa may result in part from its ability to increase dopamine (DA) levels in the substantia nigra (SN). Moreover, when striatal (ST) levels decline, elevated DA levels in the SN may be able to maintain L-Dopa-induced circling behaviour by activating D_1 DA receptors in the SN. In order to test this possibility, the effects of L-Dopa on DA levels in the SN and intranigral administration of the D_1 antagonist SCH 23390 on rotation were determined in an animal model of Parkinson's disease. Rats unilaterally lesioned in the SN with 6-hydroxydopamine (6-OHDA) that turned at least 900 times (4 h period) after a carbidopa and L-Dopa (25 mg/kg, i.p. each) treatment were included in the study. One h after this treatment, DA was elevated in the striatum and the SN ipsilateral to the 6-OHDA lesion relative to vehicle controls. However, at 2 and 3 h the DA content in the ipsilateral ST was reduced by half while the levels in the 6-OHDA-lesioned-SN were the same as that at 1 hour. Interestingly, the infusion of 1.5μg SCH 23390 in 1μl of water into the lesioned SN 20 min prior to the L-Dopa injection was able to abolish rotation that would normally have occured 2 h after L-Dopa. These results suggest a time-dependent interplay between ST and SN in the regulation of L-Dopa-induced rotation. (Supported by the Parkinson's disease foundation of Canada and the MRC).

526.1

CROSS REACTIVITY OF BRAIN REACTIVE AUTOANTIBODIES. C.S. Madsen* and S.A. Hoffman (SPON: D. Glanzman). Dept. of Microbiology, Arizona State University, Tempe, AZ 85287.

Autoantibodies to brain membrane antigens have been demonstrated in patients and in murine models of SLE. We have been characterizing the nature and specificity of these autoantibodies to determine what role, if any, they play in the neuropsychiatric manifestations of autoimmune disease. In this study we used absorption, gel electrophoretic and immunoblot techniques to examine the degree to which these autoantibodies bind uniquely to brain membrane proteins as opposed to being cross reactive with membrane antigens of other organs. Aliquots of sera from mice were absorbed with dissociated cells of brain, liver, kidney and spleen from the animal the sera was collected. These, as well as unabsorbed sera, were reacted against integral brain membrane protein of a non-autoimmune mouse strain (Balb/C). The membrane proteins were isolated by detergent extraction and phase separation, separated on SDS polyacrylamide gels under non-dissociating conditions and transferred onto nitrocellulose membrane. Sera incubated with the blot and stained with anti-mouse Ig antibody indicate that autoantibodies to brain specific as well as cross reactive antigens can be found. The data and their implications for CNS involvement in autoimmune disease will be discussed.

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526.2

A BRAIN SPECIFIC ANTIGEN IS RECOGNIZED BY ANTIBODIES IN THE SERUM OF PATIENTS DISPLAYING CNS-SLE. Daniel Rosenbluth*, D.S. Kohtz*, M. Horowitz*, H. Spiera* and S. Puszkin* Departments of Pathology & Medicine, Mount Sinai Sch. of Med., C.U.N.Y., New York, N.Y. 10029

An antibody was identified in the serum of a patient with Systemic Lupus Erythematosus (SLE) and CNS manifestations which specifically bound to a brain synaptic plasma membrane antigen. This antigen (Lp50), which appears to be an integral brain membrane glycoprotein, was not detected in extracts of cardiac and striated muscle, liver, kidney or pancreas. The serum was screened by Western blotting with LP50 purified from brain synaptic plasma membrane. Serum samples from 33 patients with connective tissue disorders including rheumatoid arthritis, polymyositis, scleroderma, vasculitis, and SLE were tested. Eleven of 21 patients who showed antibody reactivity with Lp50 had CNS disease manifested by depression, psychosis or seizures. The 12 remaining patients and 3 normal controls did not express anti-Lp50 antibodies, and had no apparent clinical CNS manifestation of disease. The physiological significance of the Lp50 antigen, and whether the antibody identified is an etiologic factor in, or just a marker of CNS disease is under further study.

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(SPON: S. Berl)

526.3

IMMUNOCYTOCHEMICAL FEATURES OF ARGYROPHILIC GRAINS, A PATHOLOGICAL ALTERATION CHARACTERIZING AN ADULT ONSET DEMENTIA DIFFERENT FROM ALZHEIMER'S AND PICK'S DISEASE H.Braak, E.Braak, I.Grundke-Iqbal* and K.Iqbal*. Dept.Anat. J.W.Goethe Univ. D-6000 Frankfurt 70, FRG, *Inst.for Basic Res. in Dev. Disabilities, Staten Island, NY 10314, USA

Brains of ten individuals afflicted with adult onset of dementia showed no neurofibrillary tangles, neuritic plaques, neuropil threads or amyloid deposits but did show argyrophilic grains scattered throughout the neuropil of some cortical areas. The grains were best demonstrated with the Gallyas silver technique for neurofibrillary changes. Most of them were spindle-shaped and reached a length of up to 9 µm and a diameter of up to 3 µm. They were quite numerous in sector CA 1 of the Ammon's horn and in layer III (pre-ß) of the entorhinal cortex. In paraffin sections, they showed immunostaining with Alz-50 and antibodies raised against paired helical filaments and a bovine-tau protein (avidin-biotin-complex-technique). No immunostaining was achieved with antibodies raised against amyloid and ubiquitin.

Some sections were processed using the chromogen 4-chloro-1-naphthol. After documentation of the immunoreactive structures the chromogen was de-stained by ethanol. The sections were then re-stained according to the Gallyas technique and showed the same structures as in the previous immunostaining.

Supported by the Deutsche Forschungsgemeinschaft.

526.4

STRUCTURAL AND FUNCTIONAL STUDIES OF THE SCRAPIE-AGENT PROTEIN. G. Bablanian, D. Windsor, P.E. Bendheim and D. Bolton. Dept. of Molecular Biology, NYS Institute for Basic Research, Staten Island, NY 10314.

Scrapie is a neurodegenerative disease of animals and is a model for the human diseases kuru, Creutzfeldt-Jacob disease and Gerstmann-Straussler syndrome. The physical properties of the scrapie agent are not known, however, a 33-37 kDa modified host protein (Sp33-37) derived from infected hamster brain is essential for disease production in lab animals. A smaller form of this protein (PrP 27-30) is produced from Sp33-37 by partial proteolysis. Chemical modification of specific amino acid residues under mild reaction conditions was used to probe the protein structure and function. The modified protein was detected using polyacrylamide gel electrophoresis and immunoblotting with polyclonal antiserum to PrP 27-30 and a monoclonal antibody, 3F4. 3F4 did not bind to the scrapie protein after cleavage at methionine residues or following modification with diethyl pyrocarbonate (DEP). Modification with succinic anhydride, *N*-succinimidyl 3-(4-hydroxyphenyl)propionate and chloramine-T did not reduce 3F4 binding. Infectivity was reduced by modification of histidine with DEP and restored after reversal with hydroxylamine, implicating histidine as an active site residue. 3F4 binding was not restored by hydroxylamine treatment. Possible sites for the monoclonal epitope are suggested from the sequence predicted from cDNA clones of Sp33-37. NIH Grant NS23948

526.5

IMMUNOPEROXIDASE LOCALIZATION OF CYTOSKELETAL PROTEINS IN SPINAL CORD OF THE MOTOR NEURON DEGENERATION (Mnd) MOUSE. **J.E. Mazurkiewicz*, L. Callahan* and A. Messer (SPON: D.A. Poulos). Dept. of Anat., Cell Biol. & Neurobiol., Albany Med. Col. and Wads. Ctr. for Labs & Res, NYS Dept. Health and Sch. of Public Health Sciences, SUNYA, Albany, NY 12208.**

We recently reported the presence of phosphorylated neurofilament (pNF) epitopes in the cell soma of spinal motoneurons of the Mnd mouse (JCB 105:316a, 1987). The monoclonal antibody we used was axon-specific; the cell body of a normal motoneuron is negative. Additional analyses of cords from animals which exhibited different degrees of symptomology showed that while only mutants contained somatic pNF, there was no apparent correlation between the number of pNF positive cell bodies and the severity of disease at the time of fixation. This may not be surprising since there is a continuous loss of spinal motoneurons with time of progression of the disease which could result in the prior disappearance of degenerating (pNF-positive) soma. Alternatively there may be an underlying Mnd neuronal alteration which is several steps removed from the symptomology. The microtubule associated protein Tau is also enriched in axons, and might also have shown an anamolous distribution in Mnd spinal motoneurons. Using an antibody to Tau-1, its localization was similar in normal and Mnd spinal motoneurons: predominantly axonal. However, in Mnd spinal cord there were a few more positive neuronal cell bodies. In addition, a large number of Tau-positive cells were observed in both gray and white matter in Mnd and normal spinal cord. The cells were small and round, and distributed throughout the tissue. A tentative identification as glia is consistent with their size, number and location. Using cell specific markers, work is in progress to positively identify these cells.

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526.6

Effects of age on the development of Huntington's-like symptoms using endogenous neurotoxin 1-Pyroglutamic acid in rats. Jennie C. Johannesen* and Richard E. Musty University of Vermont Burlington, VT 05403

A recent study has shown that 1-pyroglutamic acid, a major intermediate in the gamma-glutamyl metabolic cycle, mimics the symptoms of Huntington's Chorea when injected unilaterally into the caudate of mice (Rieke, Scarfi & Hunter, 1984).

We examined the effects of age on the development of Huntington's-like symptoms by performing bilateral injections of 1-PGA into the caudate of 6 month old and 2 year old male Wister rats. Several behavioural measures were taken, including two tests of muscle strength, a measure of aggression and general behavioural observations. In addition, the rat's footprints were examined in a straight runway to check for signs of ataxia, or gait disturbance.

We found several significant differences between young and old rats in response to bilateral lesions. Younger animals displayed significantly increased aggression while older animals displayed more ataxias & general gait disturbances. In addition, the majority of the older rats demonstrated a significant loss of muscle strength following injection. These results suggest that the neurotoxic effects of 1-PGA become more pronounced with age. This may prove helpful when examining the slow progressive degeneration of neural tissue in Huntington's patients.

526.7

IMMUNOLOGICAL REACTIONS IN CHRONIC DEGENERATIVE NEUROLOGICAL DISEASE. S. Itagaki*, P.L. McGeer, S.-G. Zhu*, H. Akiyama*, and E.G. McGeer (SPON: J. Weinberg) Kinsmen Lab, Dept Psych Univ of British Columbia, Vancouver, B.C., Canada, V6T 1W5

Many chronic degenerative neurological diseases are characterized by the appearance of a low grade inflammatory response in regions selective for particular disease. Alzheimer's, Pick's, Parkinson's and Huntington's disease, amyotrophic lateral sclerosis, Shy-Drager syndrome, progressive supranuclear palsy, and Parkinsonism-dementia of Guam are examples. The most prominent immunological finding in these diseases is reactive microglia positive for the MHC Class II glycoprotein HLA-DR, which is essential for presentation of foreign antigen to T-lymphocytes. Reactive microglia are also weakly positive for leucocyte common antigen (LCA). LCA positive round cells can be found in vessels and in the matrix of affected areas. Subpopulations of these cells, which can be identified by markers for lymphocytic subsets include T-cytoxic suppressor and T-helper/inducer cells. Reactive astrocytes, also present in these conditions, are a separate population from the microglia as shown by double immunostaining for glial fibrillary acidic protein and HLA-DR. The results suggest that cell-mediated immune responses occur in many diseases where such involvement has not previously been suspected. HLA-DR staining may be a valuable addition to standard neuropathological methods.

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526.8

ASTROCYTES APPEAR DISTINCT FROM HLA-DR-POSITIVE CELLS IN MULTIPLE SCLEROSIS PLAQUE REGIONS. E.A.Boyle, S.Itagaki and P.L.McGeer, Kinsmen Lab, Dept Psychiatry, Univ. of British Columbia, Vancouver, B.C. Canada. V6T 1W5.

It is believed that many cell-mediated immune responses depend on antigen presentation in conjunction with HLA-DR. Yet it is still unresolved which cells carry out this function in multiple sclerosis (M.S.). We have performed double immunostaining on paraformaldehyde-fixed M.S. plaques using a monoclonal antibody against HLA-DR as the putative antigen-presentation marker, and a polyclonal antibody against glial fibrillary acidic protein (GFAP) as a marker for reactive astrocytes. Other pathological features were confirmed with Luxol Fast Blue for demyelination and a monoclonal antibody against leukocyte common antigen for perivascular cuffs. Examination of stained sections showed that HLA-DR is vigorously expressed in the inflamed margins of the plaques by cells showing the typical morphology of mature macrophages and some leukocytes and, near their edges, by reactive microglia. GFAP-positive astrocytes are frequently seen in the vicinity, but we have been unable to obtain convincing evidence that they overlap with HLA-DR-positive cells. Our evidence suggests that astrocytes display HLA-DR weakly, if at all, in M.S. plaques, and that macrophages and microglia constitute the overwhelming majority of HLA-DR-positive cells.

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526.9

A PRIMATE MODEL OF HUNTINGTON'S DISEASE: UNILATERAL EXCITOTOXIC LESIONS OF THE CAUDATE-PUTAMEN AND NEURAL TRANSPLANTATION IN THE BABOON. O. Isacson, P. Hantraye*, D.Riche* and M.Maziere*. Dept. of Anatomy, University of Cambridge, Cambridge CB2 3DY, England and Frederic Joliot Hospital, Dept of Biology, CEA, Orsay 91406, France.

In the rat, many of the neuropathological and neurochemical features of Huntington's disease (HD) can be replicated by intrastriatal infusion of excitotoxins such as ibotenic acid. We have developed a lesion model of HD in the primate by excitotoxic lesions of the caudate-putamen complex (CPU) in order to test treatment strategies such as neural transplantation and pharmacological interventions. Ten monkeys received stereotaxic unilateral infusion of ibotenic acid into the right CPU, while 4 unoperated animals served as controls. Three animals received stereotaxic implantation of fetal cell suspensions prepared from striatal rat primordia into the lesioned CPU. These monkeys were treated with the immunosuppressant drug cyclosporine. Two to 35 weeks after the lesion the animals were assessed in their home-cages for spontaneous behaviours and motor abnormalities induced by various doses of the dopamine agonist drug apomorphine (0.5, 1.0 and 2.0 mg/kg). Behavioural studies indicated few spontaneous abnormalities following the unilateral ibotenic acid lesion, but injection of the dopamine agonist drug induced dramatic dyskinesias and motor stereotypies in the lesioned animals, while controls were either not affected or showed mild stereotypic motor behaviours. In vivo PET studies indicated clear lesion-induced changes in the CPU, such as reduction of dopamine receptor binding. Anatomical and histological analysis showed striatal lesions that corresponded closely to the anatomical distribution of CPU damage seen in HD. Results in progress describing the effects and survival of the transplants will be presented at the meeting.

REGULATION OF AUTONOMIC FUNCTION III

527.1

LIGHT AND ELECTRON MICROSCOPICAL OBSERVATIONS OF ACETYLCHOLINESTERASE REACTION IN DEVELOPING RAT RETROPERITONEAL PARAGANGLIA. Liisa Eränkö, Marketta Ahonen and Nils Bäck. Department of Anatomy, University of Helsinki, Finland.

The main retroperitoneal paraganglia of the newborn rat consists of different kinds of catecholamine cells; one with moderate immunofluorescence of tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH) resembling sympathetic nerve cells; another with bright fluorescence to TH and DBH. The latter type are smaller and are considered as paraganglion type cells.

The developing paraganglion cells initiated catecholamine synthesis on the 13th prenatal day. At this stage, some of these cells showed AChE reactivities. During later development, two subpopulations of cells were distinguished. One showed intense TH immunoreactivity but no AChE reactivity. The other was moderately reactive to TH and showed intense AChE staining. Electronmicroscopically these two cell populations were clearly distinguished. AChE reactivity was observed only in the neuron-like cells, in which the reaction was mainly located in the endoplasmic reticulum.

527.2

BULLFROG AUTONOMIC GANGLION CELLS HAVE LOCAL AXON COLLATERALS. C.J. Forehand and L.M. Konopka*. Dept. of Anatomy and Neurobiology, Univ. Vermont Col. Med., Burlington, VT 05405.

Autonomic ganglia in the frog are traditionally described as simple relay stations. However the variety of peptides found in ganglion cells and their preganglionic inputs suggests that these ganglia may be more complex. In an effort to define the microenvironment of identified cells after studying their responses to particular peptides, we began to label the cells by intracellular injection with HRP. We found that the morphology of both sympathetic lumbar chain cells and parasympathetic cardiac ganglion cells in the frog is more complex than the simple unipolar description normally given.

Most HRP-labeled cardiac ganglion cells (located in the interatrial septum) had extensive axon collaterals arising from the axon within 30 um of the cell bodies. These local collaterals were in addition to the main axonal projection into the cardiac muscle and contained numerous synaptic bouton-like swellings. Local axon collaterals, though less extensive than those on cardiac ganglion cells, were also present on 30% of the cells in lumbar sympathetic ganglia. The cells with local axon collaterals were a subset of the total population of ganglion cells based on size: The largest cells did not have axon collaterals. (PHS RO123978 & NSF BNS 8605611)

527.3

UNEXPECTED AUTONOMIC NERVOUS SYSTEM CONNECTIONS REVEALED BY THE HORSERADISH PEROXIDASE METHOD. J.C. Liu,* K.-P. Shaw and J.I. Yau.* (SPON:C.Y. Wen) Dept. Biol. Anat., Natl. Def. Med. Ctr., Taiwan, R.O.C.

New autonomic nervous system connections from the contralateral superior cervical ganglion (SCG) and middle cervical ganglion (MCG) to SCG were demonstrated by applying the Horseradish peroxidase (HRP) retrograde tracer technique. HRP was injected into the unilateral SCG in 12 cats. After survival times of 2 or 3 days, the animals were sacrificed and their spinal cord, brain stem and sympathetic ganglia were processed to visualize HRP labeling by using the tetramethyl benzidine method. Labeled neurons were found not only in ipsilateral T1-T5 spinal cord segments, MCG and Stellate ganglion, but also within the contralateral SCG and MCG. Lesions in the sympathetic chain caudal to the SCG injection site prevented spinal cord labeling, but not contralateral SCG and MCG labeling. Thus the contralateral pathway utilizes a rostral course. In conclusion, the presence of the contralateral projection to the sympathetic cervical ganglion challenges the principle of the two-neuron-chain peripheral autonomic nervous system. (Grant NSC 77-0412-B016-32, Taiwan, R.O.C.)

527.4

ORIGINS OF THE RENAL INNERVATION IN THE MONKEY, *MACACA FASCICULARIS*. S. F. Echtenkamp, C. F.Marfurt, and M. Jones*. Departments of Anatomy and Physiology, Indiana University School of Medicine, Northwest Center for Medical Education, Gary, IN 46408.

The origins of the renal afferent and efferent innervations in five monkeys were studied by using the retrograde transport of horseradish peroxidase (HRP). The cut ends of the right renal nerves were soaked for one hour in a solution of 15% HRP and 2% HRP-WGA and the animals were sacrificed 72-96 hours later. Labeled sensory neurons (196-543 per animal) were found in ipsilateral dorsal root ganglia (DRG) T8-L4, with the majority (57%) located in DRG T13. Ninety-two percent of the labeled afferent perikarya were found between T12 and L1. The labeled DRG cells ranged from18-64um in diameter, with a mean of 32.4um. Labeled cells were never seen in any contralateral DRG, and with a single exception in one animal, no labeled neurons were observed in the nodosal ganglia. The contributions to the renal efferent innervation from the prevertebral and paravertebral (sympathetic chain) ganglia varied considerably from animal to animal. Collectively, 57.4% of the total sympathetic innervation (5,574-14,565 cells per animal) arose from topographically organized areas of the ipsilateral prevertebral (coeliac, adrenorenal, and renal) ganglia, whereas 42.6% arose from ipsilateral sympathetic chain ganglia (T10-L3). The majority of labeled sympathetic chain cells (97%) were located in T13-L3, and of these 69% were found in L1 and L2. No labeled neurons were found in any contralateral prevertebral ganglia and only rarely (4 cells in one animal) were any cells observed in the contralateral sympathetic chain ganglia. These results reveal for the first time the origins of the renal afferent and efferent innervations in a primate. (Supported by NIH grants HL37223 to S.F.E. and 507RR5371 to C.F.M.)

527.5

APPARENT PLASTICITY OF AUTONOMIC NERVES: STIMULATION OF THE HYPOGASTRIC NERVES ELICITS INCREASES IN PENILE PRESSURE AFTER CHRONIC INTERRUPTION OF THE SACRAL PARASYMPATHETIC OUTFLOW. M.P. Olmsted*, G. Walton* and W. G. Dail. (SPON: E. Reyes) Dept. of Anatomy, Univ. of New Mexico, Sch. of Med., Albuquerque, NM 87131.

Investigations have previously documented that preganglionic fibers in the pelvic nerve and hypogastric nerve may participate in erection in mammals. The effects of stimulation of autonomic nerves on pressure in the corpora cavernosa penis (CCP) were studied in intact rats and in rats in which the pelvic nerves were unilaterally or bilaterally transected one to six weeks earlier. An increase in penile pressure in control animals occurred with stimulation of the pelvic nerve and with stimulation of postganglionic fibers from the pelvic plexus. A more consistent rise in pressure occurred with stimulation of postganglionic fibers. However, no increase in penile pressure was obtained even with bilateral stimulation of the hypogastric nerves. One week following interruption of the pelvic nerve, stimulation of the hypogastric nerve resulted in a small but significant rise in pressure in the CCP (average rise of 13mm Hg). In animals with bilateral interruption of the pelvic nerve, stimulation of either hypogastric nerve elicited an increase in pressure while in the unilaterally lesioned group, tumescence occurred only after stimulation on the lesioned side. It is hypothesized that chronic interruption of the pelvic nerves is a stimulus for compensatory changes in the penile vasodilator pathway of the hypogastric nerves. A similar mechanism may underlie the preservation of penile erection in injury to the terminal portion of the spinal cord in man. Supported by NIH RO1NS1983-5 and RR08139-12.

527.6

AUTONOMIC INNERVATION OF THE ANOCOCCYGEUS MUSCLE OF THE RAT. W. G. Dail, D. S. Trujillo*, Y. Carrillo*, N. Minorsky, D. de la Rosa* and G. Walton*. Dept. of Anatomy, Univ. of New Mexico, Sch. of Med., Albuquerque, NM 87131.

The anococcygeus muscle of the male rat is often used as a model to investigate neural control of smooth muscle. Norepinephrine is an excitatory neurotransmitter to this muscle, yet relatively little is understood of the nature of the inhibitory neurotransmitter and the location of ganglionic synapses. To investigate these questions, ganglion cells were located by injection of the retrograde tracer fluorogold into the anococcygeus. In addition, the anococcygeus muscle was stained for the presence of nerves containing acetylcholinesterase (AChE) and fibers immunoreactive for vasoactive intestinal polypeptide (VIP). Labeled ganglion cells were found in the lumbosacral sympathetic chain (average of 120), the pelvic plexus (aver. 300) and the inferior mesenteric ganglion (aver 15). Neurons in the pelvic plexus were found in the major pelvic ganglion and all along the main penile nerve to the anococcygeus muscle. Some ganglion cells were present in the anococcygeus muscle. The anococcygeus muscle has a rich plexus of AChE positive fibers and a somewhat less dense innervation by VIP-IR fibers. Immunocytochemistry of identified neurons indicates that the pelvic plexus is one source of the VIP innervation of the anococcygeus. Previous studies have failed to find an AChE+ innervation of this muscle. The presence of AChE+ fibers and VIP-IR fibers raises the possibility that acetylcholine and VIP may participate in the innervation of the anococcygeus muscle. Supported by NIH grant RRO8139-12.

527.7

AFFERENTS IN THE PELVIC VISCERA OF FEMALE RATS LOCALIZED BY ANTEROGRADE TRANSPORT OF HRP. D. M. Nance, J. Burns*, C. M. Klein* and H. W. Burden. Dept. of Anat., Dalhousie Univ., Halifax, Nova Scotia, B3H 4H7, Canada, and Dept. of Anat. & Cell Biol. East Carolina Univ. School of Med., Greenville, N.C. 27858.

Innervation of the female reproductive system provides an important signal for several neuroendocrine reflexes in the rat. Afferent feedback from the gonads may be involved in the control of the gonads and pituitary hormone release. Retrograde tract tracers indicate that the ovary receives an afferent input from thoraco-lumbar dorsal root ganglia (DRG). Diffusion following injections into the ovary or related nerves may overestimate the extent of afferent innervation. We re-examined the afferent input to pelvic viscera by means of the antero-grade transport of HRP from thoracic, lumbar and sacral DRG and tested the effects of unilateral removal of the DRG on the substance P (SP) containing fibers in the ovary, oviduct and uterus. The T_{13} and L_1 DRG provide innerva-tion to the uterine horn, oviduct and ovary and the L_6 and S_1 DRG provide af-ferent fibers to the cervix ,vagina, bladder and rectum. Innervation of the oviduct was more extensive than the uterus and only 2 /14 injections into the T_{13} and L_1 DRG produced labeled fibers in the ovary, but labeled fibers were seen in the ovarian bursa. Injections into the L_6 and S_1 DRG produced many labeled fibers in the cervical portion of the uterus, vagina, base of the bladder and rectum. Innervation of the vagina was greater than the cervix. Relative distribution of SP-like fibers was: oviduct > uterine horn > ovary. Removal of the T_{13} and L_1 DRG eliminated or reduced the number of SP-like fibers from these viscera on the same side as the surgery. Thus, cranial structures receive a modest and diffuse afferent supply from the thoraco-lumbar DRG and the caudal part of the reproductive tract, bladder and rectum receive an extensive afferent supply from the lumbo-sacral DRG. There is a modest afferent input to the ovarian bursa and surrounding tissue, but a limited number of afferent fibers enter the gonad. Supported by M.R.C. of Canada.

527.8

DISTRIBUTION AND CHARACTERIZATION OF PELVIC NEURONS WHICH PROJECT TO THE BLADDER, COLON OR PENIS IN RATS. A.M. Booth, J.R. Keast, C.R. Murray* and W.C. de Groat. Dept. Pharmacology, Univ. Pittsburgh, Pittsburgh, PA 15261.

The distributions of pelvic neurons which supply three organs of male rats were defined by using retrogradely transported dyes, injected into the bladder, colon or penis and removing the major pelvic ganglia (MPG) 3 days to 6 weeks later. For each organ there was a different distribution of labelled neurons: those supplying the bladder were located throughout the MPG, those to the colon located mainly dorsally, close to the entrance of the pelvic nerve and those to the penis mainly found near or within the penile nerve. Immunoreactivity for vaso-active intestinal peptide (VIP), neuropeptide Y (NPY), tyrosine hydroxylase (TH), leu-enkephalin (LENK), somato-statin (SS) or cholecystokinin (CCK) was identified in dye-labelled neurons and their surrounding nerve terminals. There were unique features for each neuronal population. Many colon and bladder neurons, but very few penis neurons, contain TH or NPY; most penis neurons, but very few colon or bladder neurons, contain VIP; some colon neurons contain LENK, but LENK was rarely seen in bladder or penis neurons; SS or CCK were found in nerve terminals around many bladder or colon neurons, but were rare around penis neurons. These features may indicate differences in neurotransmission to each organ. (J.K. is supported by an NH&MRC C.J. Martin Fellowship.)

527.9

REORGANIZATION IN CAT PELVIC PLEXUS FOLLOWING PARASYMPATHETIC PREGANGLIONIC DENERVATION. S. Smerin, A.M. Booth, M. Kawatani & W.C. de Groat. Depts. of Pharmacol. & Behav. Neurosci. Univ. of Pittsburgh, Pittsburgh, PA 15261

Chronic resection of the parasympathetic preganglionic innervation to the pelvic plexus results in the evolution of new patterns of urinary bladder (UB) activity and neural firing on UB postganglionic nerves in response to pelvic (PN) or hypogastric (HGN) nerve stimulation. These patterns include: 1) sustained UB contractions evoked by stimulation of the HGN, and 2) increased asynchronous neural activity in UB postganglionic nerves following a tetanizing stimulus to the lesioned PN. Conventional in vitro intracellular recording techniques were used to examine the synaptic correlates of these events more than one year after resection of the sacral dorsal and ventral roots in the adult cat. Lucifer Yellow was injected into most cells to examine their morphology.

These recordings revealed: 1) a high percentage of cells without synchronously evoked fEPSPs, 2) fewer inputs to innervated cells that were of abnormally long latency and large amplitude, 3) an evoked asynchronous discharge in the majority of cells, 4) different thresholds for fEPSPs and asynchronous discharge, and 5) abnormal cell morphology. These results confirm significant synaptic reorganization within bladder ganglia of the cat following partial denervation.

527.10

INFLUENCE OF SACRAL AFFERENT INPUT AND RECURRENT INHIBITION ON PONTO-SPINAL MICTURITION PATHWAYS. B. Mallory, M. Kruse, J. Roppolo, H. Noto and W. deGroat. Depts. of Pharmacol. & Behav. Neurosci., Center for Neurosci., Univ. of Pittsburgh, Pittsburgh, PA 15261.

We have assessed the effect of inputs that are known to influence spontaneous contractions of the urinary bladder (C-UB) on C-UB elicited by electrical stimulation (ES) of the pontine micturition center (PMC). ES of the proximal cut S2 ventral root (2V, 20 Hz, 0.05 msec) or mechanical stimulation of the rectum inhibited both spontaneous C-UB and C-UB elicited by PMC stimulation. However perigenital (PG) stimulation which inhibited spontaneous C-UB, facilitated C-UB elicited by PMC stimulation. Section of dorsal roots L7 through S3 abolished spontaneous C-UB but abolished neither the C-UB nor the relaxation of the external urethral sphincter (EUS) elicited by ES of the PMC (90-100 uA, 0.3 msec, 300 msec trains at 50 Hz). C-UB elicited by PMC stimulation in the deafferented animal, were not affected by neuromuscular blockade but were abolished by section of the sacral ventral roots. We conclude that the UB/EUS synergy during micturition is organized within the CNS independent of sacral afferent input and that recurrent inhibitory, rectal inhibitory and PG facilitatory inputs modulate the descending PMC pathway whereas PG afferent inhibitory input likely acts on the afferent arc of the spinobulbospinal micturition reflex.

527.11

ULTRASTRUCTURAL ANALYSIS OF ENKEPHALINERGIC TERMINALS IN PARASYMPATHETIC GANGLIA (PSG) INNERVATING THE URINARY BLADDER OF THE CAT. M. Kawatani, S. Shioda*, Y. Nakai*, C. Takeshige* and W.C. de Groat. Depts. Physiol. and Anat., Showa Univ., Tokyo, and Depts. Pharmacol. and Behav. Neurosci., Univ. Pittsburgh, Pittsburgh, PA 15261.

Previous studies revealed that leucine-enkephalin (LENK) was present in sacral preganglionic neurons (SPGN) which innervate the urinary bladder and in varicosities surrounding postganglionic neurons in bladder PSG. The varicosities were eliminated after sacral ventral root transection indicating that they were terminals of SPGN. Present studies examined the ultrastructural characteristics of LENK terminals in the PSG. Experiments were conducted in 7 adult cats using standard EM-immunohistochemistry. LENK was identified only in axons and terminals in PSG. LENK positive terminals exhibited large dense core vesicles and small clear spherical vesicles. LENK-IR was primarily associated with the large dense core vesicles. The average size of the large dense core vesicles was 105 nm whereas small clear vesicles averaged 50 nm. LENK terminals made synaptic contact with the dendrites and soma of the principal neurons. Axo-axonic contacts were identified in few cases. The synapses were of the symmetrical type. These observations suggest that LENK is a co-transmitter with acetylcholine in the sacral preganglionic outflow of the cat.

527.12

THE EFFECTS OF SUPERFICIAL CERVICAL SPINAL STIMULATION ON SYMPATHETIC PREGANGLIONIC NEURONS. L.P. Schramm, M.P. Gilbey, and J.P. O'Reilly*. The Johns Hopkins School of Medicine. Baltimore, MD 21205

Experiments were conducted to determine whether superficial cervical stimulation inhibits glutamate-elicted activity of otherwise silent sympathetic preganglionic neurons (SPN), indicating the direct impingement of the dorsal spinal sympathoinhibitory system (DSSS) on SPN. Three-hundred gram, male, Sprague-Dawley rats were anesthetized with chloralose and prepared for recording from SPN at the T2-T3 levels. These neurons were identified by antidromic stimulation from the cervical sympathetic chain. Recordings were made through one barrel of a three-barrel electrode from which glutamate could be iontophoresed. Stimulus-elicited inhibition of sympathetic activity was manifested by depressor responses or by decreases in renal nerve sympathetic activity. Of the 20 SPN recorded, 16 exhibited activity only during glutamate iontophoresis. Very low (but not higher) rates of glutamate-elicited firing could be inhibited by cervical stimulation in 6 of these otherwise-silent SPN. Inhibition was well-sustained in 3 of these neurons, but lasted only a few seconds in 3. Glutamate-elicited activity in the remaining 10 SPN was either unchanged or increased by cervical sympathoinhibitory stimulation. Cervical stimulation elicited well-sustained inhibitions of action potentials of all 4 spontaneously active SPN. Because inhibition of SPN by cervical stimulation appeared to be very weak, we tentatively conclude that this inhibition acted by reducing subthreshold excitation of glutamate-excited SPN, not by directly inhibiting them. Whether inhibition of SPN generating abdominal sympathetic activity will be more powerful remains to be tested.
Supported by NIH Grant HL16315.

527.13

AN EXTRACELLULAR STUDY OF THE RESPONSES OF LUMBAR SYMPATHETIC PREGANGLIONIC NEURONES TO NOXIOUS STIMULATION OF THE SKIN IN THE CAT. M.P. Gilbey, J.F.R. Paton* and I.M. Clark*, Department of Physiology, Royal Free Hospital Medical School, Rowland Hill Street, LONDON NW3 2PF, U.K.

Response patterns of lumbar sympathetic preganglionic neurones (SPN) to noxious stimulation of the skin have been studied previously using single fibre recordings (Janig, W., Rev. Physiol. Biochem, Pharmac., 102, 119-213, 1985). In this study extracellular recordings from SPN have been made as the technique permits cells to be driven pharmacologically (Gilbey, M.P. et al, J. Physiol. 378 : 253-265, 1986) and therefore inputs to 'silent' SPN as well as those having ongoing activity can be studied.

Forty six neurones have been studied in chloralose anaesthetized cats. These were identified antidromically by stimulation of the lumbar chain between L5 and L6 ganglia. Of 6 glutamate-activated SPN tested for their response to noxious input (heat 50-60°C), 2 ceased to discharge, one had a slightly decreased discharge during the stimulus and a rebound excitation, and 3 were unaffected. One neurone having ongoing activity was unaffected by the stimulus.

These results show that the discharge of glutamate-activated SPN can be influenced by noxious stimulation of the skin.
Supported by the British M.R.C. and British Heart Foundation*.

527.14

Enkephalinergic Projections from Brainstem to the Spinal Cord. MA Romagnano, R Harshbarger, CB Saper, and RW Hamill. Neurology Dept., Univ. of Rochester/Monroe Community Hosp., Rochester, NY, 14603 and Dept. Pharm. & Physiology, Univ. of Chicago, Chicago, Il, 60637.

In the rat, enkephalinergic (Enk) fibers in the spinal cord are found in a ladder-like pattern corresponding with preganglionic sympathetic nuclei. These Enk fibers are of both supraspinal and intraspinal origin. We combined immunohistochemistry for Enk in the brainstem with retrograde transport of FB unilaterally injected into the intermediolateral cell column at the junction of spinal levels T2 and T3.

Double-labeled neurons are found ipsilaterally and contralaterally in many areas of the brainstem previously shown to project to the spinal cord including the rostroventrolateral medulla (C1 area) and the region dorsal to the inferior olive (B3 area). In the pons and midbrain double-labeled neurons are located in the same regions as noradrenaline cells and fiber pathways. These double-labeled cells are the source of brainstem Enk projections to the spinal cord and may represent the origin of an opioid influence on autonomic functions.

527.15

IDENTITY OF VAGAL AFFERENTS AND EFFERENTS IN RELATION TO NEURONS CONTAINING ENKEPHALIN-LIKE IMMUNOREACTIVITY. J. Chan, L. Velley, T.A. Milner, S. Morrison, and V.M. Pickel. Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Immunoautoradiographic localization of an antiserum directed against Met^5-enkephalin was examined in relation to sensory and motor components of the rat dorsal vagal complex. The afferents and efferents were identified by anterograde and retrograde transport of horseradish peroxidase applied in a cuff around the proximal end of the vagus nerve sectioned at the cervical level. By light microscopy, enkephalin-like immunoreactivity (ELI) was seen as silver grains diffusely distributed throughout the neuropil and in isolated perikarya located within the medial nuclei of the solitary tract (m-NTS). Portions of the m-NTS containing ELI also contained extensive anterograde labeling of vagal afferents. Conversely, the motor nuclei of the vagus contained retrogradely labeled perikarya and proximal processes. Motor neurons were surrounded by silver grains indicative of ELI. By electron microscopy, ELI was associated principally with large dense core vesicles in axon terminals. Terminals containing ELI were detected in both sensory and motor portions of the vagal complex as identified by anterograde labeling of axon terminals or retrograde labeling of perikarya and dendrites. These results suggest that opioids may be important modulators of both sympathetic and parasympathetic reflexes involving the dorsal vagal complex. (Supported by NIH grants MH00078 and HL18974 (VMP) and INSERM 856024 (LV)).

527.16

INNERVATION OF THE DORSAL MOTOR NUCLEUS OF THE VAGUS IN THE CAT: IMMUNOCYTOCHEMICAL AND RETROGRADE TRACING EVIDENCE FOR PROJECTIONS FROM HINDBRAIN, BUT NOT FOREBRAIN NUCLEI. R.A. Gillis* E.K. Friedman* W.P. Norman and P.J. Hornby*. Depts of Pharmacology and Anatomy, Georgetown University. Washington, DC.

It is well documented that a number of forebrain and hindbrain nuclei project to the dorsal vagal complex [i.e. the area postrema (AP), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMV)]. Use of immunocytochemistry and antibodies to thyrotropin-releasing hormone (TRH), neuropeptide Y, neurotensin, oxytocin and substance P reveals differences in the distributions and densities of these neuropeptides in the nuclei of the dorsal vagal complex. Specifically, a marked density of TRH puncta is noted within the DMV, whereas few TRH puncta are observed in the NTS and AP. The region of the DMV with the most dense TRH innervation is 1-3mm rostral to the obex and contains vagal neurons projecting to the stomach. Microinjection or iontophoresis of HRP-WGA (10%) or fluorogold (2%) into the "gastric" region of the DMV, results in labelled cell bodies in the ventrolateral medulla and raphe nuclei but not in the paraventricular nucleus of the hypothalamus (PVN), although microinjections which spread into the NTS did label cells in the PVN. These data suggest that TRH projections to the DMV arise from neurons in the raphe nuclei and not the PVN. A role of these projections in control of gastric motility is supported by the fact that microinjection of 100-200nl of 0.5M glutamate into the raphe obscurus results in an increase in gastric motility. (Supported by NIH grant AM 29975)

527.17

IDENTIFICATION OF THE TERMINALS OF VAGAL EFFERENT FIBERS AND THEIR APPARENT TARGET NEURONS IN THE RAT GUT. A. L. Kirchgessner and M. D. Gershon. Dept. of Anatomy & Cell Biology, Columbia Univ. P&S, New York, NY 10032

Although the intrinsic innervation of the bowel is independently capable of mediating reflexes, the gut receives a CNS innervation from the dorsal motor nucleus of the vagus (DMX). Since the number of efferent vagal fibers is small, it is not clear how the vagus affects enteric activity. The current experiments were undertaken as a partial test of the hypothesis that the CNS innervates a small number of command neurons in enteric ganglia. The anterograde tracer, *Phaseolus vulgaris* leucoagglutinin (PHA-L), was iontophoretically injected into the DMX and 10-21 days later, PHA-L was immunocytochemically visualized in the bowel. Varicose vagal efferent fibers, labeled by PHA-L, were found in the myenteric plexus as far distally as the ileo-colic junction. These axons were relatively rare, entered a minority of myenteric ganglia, and contacted a small proportion of the neurons. Vagal efferent fibers were most numerous in the gastric antrum and became progressively more sparse distally. Possible target neurons were neurochemically identified by simultaneously demonstrating the immunoreactivities of VIP, enkephalin, galanin, 5-HT, and tyrosine hydroxylase (TH) along with that of PHA-L. Vagal terminals appeared to contact VIP+ and 5-HT+, but not galanin+ or enkephalin+ neurons. Vagal efferents were galanin+, but TH-; therefore, some vagal preganglionic fibers probably contain galanin; however, TH-containing axons in the vagus nerve are likely to be of sympathetic origin. Following injections of Fluoro-Gold (FG) into the stomach, a subset of cells in the DMX labeled by retrograde transport of FG were galanin+. Galanin+ terminals also encircled FG-labeled DMX cells. These observations are consistent with the hypotheses that the vagus nerve innervates a small number of command neurons in the myenteric plexus, that some of these neurons contain VIP or 5-HT, and that galanin may be a modulator of CNS control of the bowel. Supported by NIH grants # NS12969, NS 07062, and the PMAF.

527.18

SYNAPTIC ORGANIZATION OF VAGAL AFFERENTS AND EFFERENTS IN THE MEDULLA OBLONGATA. M. Kalia. Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107.

Vagal afferent fibers terminate in the nucleus of the tractus solitarius (nTS) with a distinct viscerotopic and modality-specific organization (Kalia & Mesulam, '80). Intraaxonal injections of HRP into functionally identified pulmonary afferents have revealed striking features of these terminals in the nTS (Kalia & Richter, '85). This study focuses on vagal afferents terminating in all the subnuclei of the nTS and determines the nature of their terminations. In addition, neurons in the dorsal motor nucleus of the vagus (dmnX) were studied in order to examine terminations on their dendrites and somata. Adult cats and rats were anesthetized and the right nodose ganglion was injected with cholera toxin-horseradish peroxidase conjugate. Following a 48-hour survival period, the animals were perfused and serial vibratome sections of the medulla oblongata were reacted for electron microscopy using tetramethyl benzidine-ammonium molybdate at pH 6.6, followed by slow osmication, pH 5.5 (Ralston, et al, '87). The nTS-dmnX complex was examined at each rostrocaudal level for reaction product under the light and electron microscope. Axon terminals of vagal afferents established contact with large dendrites, including dendritic shafts and spines. In addition, axo-axonal contact with axon terminals containing pleomorphic vesicles were found. Somata of dmnX neurons were surrounded by medium-sized dendrites, however, no contact with these unlabeled dendrites was found in the sample examined. Dendrites of dmnX cells were contacted frequently by axon terminals. (Supported by USPHS HL30991, HL33632, HL40270.)

527.19

SUBSTANCE P AND GABA CONTRIBUTE TO THE CONTROL OF AUTONOMIC FUNCTION BY LAMINA I-II NEURONS OF TRIGEMINAL N. CAUDALIS. D.A. Bereiter, A.Benetti* and D.S.Gann Depts. Surgery & Neurobiology, R.I.Hospital/Brown Univ., Providence, R.I. 02902.

Excitation of neurons, by L-glutamate, within specific laminae (I-II or V-VI) of trigeminal n. caudalis (Vc) evokes endocrine and autonomic responses consistent with the activation of nociceptors from orofacial regions. To assess the effects of putative neurotransmitters within Vc on these reflex responses, microinjections (40 or 160nl) of substance P (SP), a GABA antagonist (bicuculline,BMI), or a GABA agonist (muscimol) were directed at various laminae of Vc in cats anesthetized with chloralose, paralyzed with gallamine triethiodide. Arterial pressure, heart rate, and expiratory CO2 were monitored continuously. Adrenal catecholamine (CA) secretion was assessed from adrenal vein plasma samples by HPLC with EC and peripheral venous plasma ACTH was measured by RIA. SP (36pmol) evoked increases in adrenal CA secretion, in ACTH, in arterial pressure, and in heart rate by +1min after injections into lamina I-II of Vc, whereas injections into more ventral laminae of Vc had no significant effects. BMI (62pmol) increased CA secretion and arterial pressure by +1min, whereas muscimol (280pmol) caused a delayed decrease in CA secretion and in arterial pressure after lamina I-II injections. BMI or muscimol did not affect significantly the release of ACTH regardless of the laminar site of injection within Vc, whereas injections of BMI or muscimol into more ventral laminae of Vc had no consistent effects on CA secretion, on arterial pressure or on heart rate. SP-evoked changes in CA secretion were not correlated with changes in adrenal blood flow, whereas BMI- and muscimol-evoked changes in CA secretion were well correlated ($P<.01$) with changes in adrenal blood flow. The data indicate an excitatory input to lamina I-II neurons by SP in the control of adrenal CA secretion and in the release of ACTH by Vc, consistent with a possible role in nociception. GABA may exert a tonic inhibitory influence on lamina I-II neurons that contribute to the reflex control of adrenal CA secretion. The identity of putative neurotransmitters that affect autonomic function via lamina V-VI neurons of Vc remain unknown. Supported by NIH #'s NS26137(DAB) & DK26831(DSG).

527.20

THE HUMAN INTERMEDIATE RETICULAR NUCLEUS (IRt). G. Paxinos, I. Törk*, G. Halliday* and W.R. Mehler. Schools of Psychology and Anatomy, Univ. New South Wales, Kensington, Australia 2033, and Dept of Anatomy, Univ. Calif. at San Francisco, Calif. 94143.

The IRt has recently been identified in the rat as a zone between the gigantocellular and parvocellular reticular nuclei which contains some large, as well as medium and small cells and is more reactive for acetylcholinesterase (AChE) than its neighbors (Paxinos and Watson, *The Rat Brain in Stereotaxic Coordinates*, Academic Press, Sydney, 1986). We now delineate the boundaries of this nucleus in the human on the basis of chemoarchitecture. The nucleus has the shape of an arc with a lateral concavity and nestles the ambiguus and retroambiguus nuclei. It commences at the pyramidal decussation and continues until the facial nucleus, and separates the dorsal from the ventral medullary reticular nuclei as well as the gigantocellular from the parvocellular reticular nuclei. Substance P positive cells and fibers are found throughout this nucleus. Catecholamine cells and fibers are also found in IRt but they are more prominent in the part ventrolateral to the ambiguus nucleus. Dorsally, outlying tyrosine hydroxylase positive cells of this nucleus are found in the cell poor zone that caps the medial pole of the dorsal motor nucleus of vagus. 5-Hydroxytryptamine containing cells are found intermingled with catecholamine cells mainly in the caudal part of IRt. This suggests that the autonomic regulatory centers localized in the ventrolateral medulla are part of a larger zone extending dorsomedially.

527.21

ANATOMICAL EVIDENCE THAT AUTONOMIC AND VISCERAL AREAS CONVERGE ON PARAGIGANTOCELLULARIS, A MAJOR AFFERENT TO LOCUS COERULEUS. *E.J. Van Bockstaele, V.A. Pieribone, G. Aston-Jones and M.T. Shipley*[1], Dept Bio & Cntr Neural Sci., New York Univ., NY 10003; [1]Dept. Anat. Cell Bio, U. Cincinnati Col. Med.,OH 45267

Recent investigations have found that the paragigantocellularis (PGi) in the ventrolateral medulla is broadly influential, being both a major afferent to the nucleus locus coeruleus (LC) and a key element in regulating the sympathetic nervous system. While previous studies have investigated afferents to PGi, we have re-examined this issue so as to focus on areas of PGi that project to LC, using recently developed, more sensitive transport techniques.

Wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) or fluoro-gold (FG) was iontophoretically injected into PGi and retrogradely labeled cells were identified in rat. WGA-HRP and FG yielded similar results, and in all WGA-HRP cases ipsilateral LC contained anterograde labeling. Our results confirm some previous findings, including projections from the nucleus of the solitary tract (NTS), A1 area, gigantocellularis, lateral parabrachialis, periaqueductal gray and lateral hypothalamus. However, projections not previously reported were also identified, including lumbosacral cord, medullary reticular field, contralateral PGi, A5 area, supraoculomotor nuc., Kolliker-Fuse nuc. (reported in cat and rabbit) and medial prefrontal cortex. Topography for these afferent terminations was investigated by varying injection placements within PGi. Retrogradely labeled cells in hypothalamus and medial prefrontal cortex occurred primarily after caudal injections in PGi. In addition, retrograde labeling in caudal NTS (commissuralis level) was most prominent following caudal PGi injections while those in rostral PGi revealed retrogradely labeled neurons in more rostral NTS areas. The present findings indicate that many areas afferent to PGi are implicated in autonomic and visceral regulation. Further studies will determine whether such afferents regulate the subset of PGi cells that project to LC; if so, these pathways may globally disseminate information pertaining to visceral and autonomic function by coordinating these functions with LC activity. Supported by NINCDS grant NS24698, ONR contract N00014-86-K-0493, and the Air Force Office of Scientific Research.

527.22

CATECHOLAMINERGIC CELLS OF THE VENTROLATERAL MEDULLA AND NUCLEUS OF THE SOLITARY TRACT INNERVATE THE CENTRAL NUCLEUS OF THE AMYGDALA IN THE RAT. A.M. Zardetto-Smith and T.S. Gray. Dept. Anat., Loyola Univ., Maywood IL 60153.

The central nucleus of the rat amygdala mediates the visceral and somatic responses associated with various adaptive behaviors, such as fear and the defense reaction. The nucleus of the solitary tract (NTS), the site of first synapse for cardiovascular afferent fibers, projects to the central nucleus. In this study, the possibilty that catecholaminergic neurons in the NTS innervate the central nucleus was examined using the combined retrograde tracer (Fluoro-Gold) and immunohistochemical (tyrosine hydroxylase) technique. Retrogradely labeled tyrosine hydroxylase-immunoreactive cells were observed in both the NTS and ventrolateral medulla. These results demonstrate that catecholaminergic neurons in both the ventrolateral medulla and NTS innervate the central nucleus. Central catecholamine neurons, the NTS and ventrolateral medulla have each been implicated in the autonomic control of blood pressure. The catecholaminergic projections from the NTS and ventrolateral medulla may convey cardiovascular interoceptive information to the central nucleus. Studies are in progress to determine if the catecholaminergic innervation of the central nucleus arises from the adrenergic (C1/C2) or noradrenergic (A1/A2) group of catecholamine neurons within the brainstem. (Supported by Sigma Xi Grants-in Aid of Research and NIH NS 20041).

527.23

STATIC MUSCULAR CONTRACTION ALTERS THE DISCHARGE FREQUENCY OF NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA IN CATS R.M. Bauer*, G.A. Iwamoto and T.G. Waldrop* (Spon: J.A. McMillan) Depts. of Physiology and Vet. Biosciences, University of Illinois, Urbana, IL.

The areas in the central nervous system which modulate the sympathoexcitatory responses to muscular contraction are not well understood. Recent studies from this laboratory have demonstrated that the reflex arterial pressure response to muscular contraction can be attenuated after bilateral microinjections of a glutamate antagonist into the rostral ventrolateral medulla (RVLM). The purpose of the present study was to determine if static contraction of hindlimb muscles alters the extracellular single unit activity of neurons in the RVLM. In anesthetized cats, contraction elicited by stimulation of L7 and S1 ventral roots evoked increases in arterial pressure, heart rate and minute volume. The majority (90%) of cells (n=50) recorded from within the RVLM (2.0 mm rostral and 3.75-4 mm lateral to obex and 4.7-5.5 mm below the dorsal surface) responded to contraction with a sustained 70% or greater increase in firing rate. The firing frequency of neurons recorded from sites dorsal and lateral to the RVLM were not altered significantly during contraction. These findings suggest that the reflex increases in cardiorespiratory activity evoked by muscular contraction involve neurons in the RVLM. (Supported by Am. Heart Assoc.)

527.24

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF ANGIOTENSIN ACTIONS IN THE AREA POSTREMA. S. Papas* and A.V. Ferguson, Dept. of Physiology, Queen's University, Kingston, Ontario, Canada.

The area postrema (AP) is a midline circumventricular organ located on the dorsal surface of the caudal medulla in the rat, overlying the nucleus of the solitary tract (NTS). It sends major projections to the NTS and the parabrachial nucleus (PBN) and contains a high concentration of angiotensin II (AII) receptors. In many species, the AP is thought to centrally mediate AII's effects on the cardiovascular system. In this study, electrophysiological techniques were used to investigate the effects of systemic AII on neuronal activity in the AP and NTS, and to examine connections between the AP and the PBN.

Recordings were obtained from neurons in the AP and NTS of anesthetized male rats and the effects of iv AII (100ng-500ng), or PBN stimulation on the activity of these neurons were examined. A total of 57 cells were recorded from in the AP. Of the 9 cells tested with systemic AII, activity was enhanced in 67% and unaltered in 33% of the neurons. Recordings were made from 30 cells in the NTS, of which 15 were tested with iv AII. The activity of 73% of the neurons was inhibited upon AII injection. Two cells (13%) exhibited a rise in activity and 13% were not affected. PBN stimulation affected the neuronal activity of 12 neurons out of the 21 cells tested. Five neurons (25%) were antidromically identified as projecting to the PBN region and 33% were orthodromically identified as projecting to the AP. These findings suggest that systemic AII exerts opposing effects on cells in the AP and the NTS. Further experiments will elucidate both the mechanism of this action and the effects of AII on neurons projecting from the AP to the PBN. Supported by the MRC of Canada.

527.25

THE SALIVATORY NUCLEI OF *MACACA FASCICULARIS*. A.R. Eden, K.J. Chandross*, J.T. Laitman* and P.J. Gannon. Departments of Otolaryngology and Anatomy, Mount Sinai School of Medicine, New York, NY 10029.

The distribution of neurons representing the superior and inferior salivatory nuclei (SSN, CN VII; ISN, CN IX) of adult macaques was determined using HRP tract tracing methods. The tympanic and chorda tympani nerves (TN, n=7; CTN, n=4) were transected in the middle ear and cannulated with a HRP (30%) filled tube. The greater petrosal nerve (GPN, n=5) was cannulated distal to the facial hiatus. After perfusion fix, 40um frozen-cut brainstem sections were processed using standard HRP-TMB methods. Labelled cells were quantified by two separate observers.

Results showed that for the **TN**, three distinct groups of neurons were labelled ipsilaterally. The largest group, situated along the dorsolateral margin of the medullary reticular formation, extended from the level of the caudal pole of nucleus ambiguus (NA) to the caudal superior olive. A scattered group of labelled cells was present around the caudal pole of NA. A medium-sized group was situated around the dorsal margin of the facial nucleus (FN). For the **CTN**, two ipsilateral cell groups were labelled. One group was located outside the dorsal boundary of the FN. The other group was located 1.3mm dorsomedial to the FN. The two groups merged rostrally to form a large dorsoventrally scattered chain of cells. For the **GPN**, two cell groups were labelled ipsilaterally. The larger group was located along the lateral margins of FN. A small column of cells was present 1.2mm dorsomedial to FN. A few scattered cells bridged the two groups.

This study demonstrated what may be numerous subnuclei of the inferior and superior salivatory nuclei. There is some overlap of cell groups for the three nerves, both around the margin of FN and more dorsally. The diversity and number of cell groups suggest that functions other than lacrimation, salivation and nasal mucous secretion may be subserved. Current studies focus on a potential common role of ISN and SSN components in middle ear function by chemoreceptor modulation. (Supported by NIH Grant NS22685)

527.26

THE TYMPANIC PLEXUS OF *MACACA FASCICULARIS*. P.J. Gannon, J.T. Laitman* and A.R. Eden. Departments of Otolaryngology and Anatomy, Mount Sinai School of Medicine, New York, NY 10029.

The tympanic plexus (TP) of the middle ear is the crossroads of three cranial nerves (CN VII, IX and X) and the autonomic nervous system. This study investigates the anatomy of the TP and the relations of a little known intrinsic component, the glomus/ganglion (G/G) cells associated with the tympanic (TN) and caroticotympanic (CTN) nerves.

In five adult animals the superior cervical ganglion (SCG) was ablated unilaterally, then the head and neck fixed by transcardiac perfusion at 7, 20, 30, 42 and 48 days. Two unoperated animals were used as controls. After 1 week post-fix the middle ear was flooded with 2% osmium tetroxide for 30min to distinguish neural elements of the TP against the promontory mucosa. After drawing and photography, TP components were dissected and processed for EM. In four animals the TN was present bilaterally as a large single nerve traversing the promontory. In three other animals a hypotympanic branch of the TN was present unilaterally. In all animals, several CTN coursed from the region of the carotid artery to join the TN. Often associated with this nerve junction were large delta-shaped G/G cell clusters. These clusters were also present at many locations throughout the TP, mostly associated with CTN. Ultrastructural analysis showed degenerating terminals synapsing on, and in close association with, these cells *bilaterally*. Degenerating myelinated and unmyelinated axons were also present in the TN and CTN bilaterally. Not all CTN axons were degenerated, suggesting a source other than SCG. Although G/G cells were associated with numerous mucosal blood vessels, no fenestrated endothelia were observed. The large G/G cells (35um) showed many somatic extensions with multiple synaptic relations, a large spherical nucleus with a single nucleolus, abundant cytoplasmic organelles, and some dense cored vesicles. They are enveloped by satellite cells with thin tortuous cytoplasmic processes, an elongated nucleus with dark clumped chromatin, and a dark organelle-poor cytoplasm.

The potential role of TP elements in subserving a middle ear aeration reflex analogous to that seen in the lower respiratory tract is the focus of present studies.

527.27

DISTRIBUTION OF VASOPRESSIN AND VASOACTIVE INTESTINAL POLYPEPTIDE IN CANINE PARAVENTRICULAR AND SUPRAOPTIC HYPOTHALAMUS. C.H. Block and H. Vilsack*. The Research Institute of the Cleveland Clinic Foundation, Cleveland, OH 44195

From initial anatomical studies, vasopressin (VP) and vasoactive intestinal polypeptide (VIP) are co-distributed in the paraventricular (PVN) and supraoptic (SON) nuclei of the canine hypothalamus (Block, 1987). However, in the normal Sprague-Dawley rat, VIP-containing cell bodies are not found in the PVN, while in the colchicine-treated homozygous Brattleboro and lactating or adrenalectomized rat, VIP immunoreactivity within perikarya of the PVN is evident (Mezey, 1986). Since the PVN and SON are significant hypothalamic regions for fluid regulation and control of pituitary and autonomic functions in the dog, the distributions of the neuropeptides, VP and VIP were examined in the canine brain.

Eight male mongrel dogs (2-6 kg body weight), 4 pretreated with colchicine, were perfused with Zamboni's fixative and brain tissue was processed for localization of VP and VIP using single- and dual- immunocytochemistry.

At the level of the optic chiasm, two distinct populations of cells containing VP- and VIP-immunoreactivity were observed in the periventricular-PVN. Additionally, many VP cells were found throughout the SON, whereas VIP-containing cells were localized to the medial SON. At mid-anterior-posterior levels of the PVN, a dense population of cells containing VP were visualized throughout the posterior magnocellular division, while VIP-containing cells were found primarily in the lateral aspect of the posterior magnocellular division of PVN. Double-stained neurons, containing VP and VIP, were scattered in the posterior magnocellular division and accessory PVN, and in the caudal aspect of the medial-SON and infundibular region.

These studies indicate a coincidence and overlap of VP- and VIP-containing cell populations in the PVN and SON of the dog. Since studies conducted in the rat suggest that VIP may participate in the release of prolactin and VP, it is plausible that, in the dog, VIP may also contribute to release of VP and fluid regulating mechanisms.

{Supported by NIH Grant HL-37927 (CHB)}

527.28

OLFACTORY INPUT TO AMYGDALOID NEURONS PROJECTING TO THE DORSAL MEDULLA. M.D. Cassell* and L. Modarressi*. (SPON: J. West). Dept. Anat., Univ. of Iowa, Iowa City, IA 52242.

The taenia tecta (TT) appear to be the main source of olfactory inputs to the central amygdaloid nucleus (Ce). The TT projection in the rat terminates in the medial Ce and is in a position to influence Ce neurons projecting to the medulla. We have therefore examined the post-synaptic relationships of TT terminals in the rat Ce at the ultrastructural level. Unilateral electrolytic lesions of the TT were made in 12 rats, 6 of which received concurrent injections of 1.5% HRP-WGA into either the NTS or parvicellular reticular formation. Peroxidase activity was detected using Co-Ni/DAB intensification of TMB chromagen. At 48 hr post-lesion, degenerating terminals were observed in the medial Ce on perikarya (12%), small (<500 nm dia) dendrites (36%) and large (>500 nm dia) dendrites (52%). No axo-spinous contacts were observed. Degenerating terminals on large dendrites were usually clustered into groups of 2-4 terminals per dendrite profile. Similar patterns of degeneration were observed on HRP labelled perikarya and large dendrites. Control injections of HRP into medulla alone produced degenerating and labelled terminals in the ventrolateral Ce but not observed in contact with retrogradely labelled neurons. The present results provide evidence of a polysynaptic route through the Ce by which olfactory influences on autonomic functions may be mediated.

527.29

CALCITONIN GENE- RELATED PEPTIDE IMMUNOREACTIVE INNERVATION OF THE INSULAR CORTEX OF THE RAT. Y. Yasui*, D.F. Cechetto, and C.B Saper, (Spon: P.A. McGrath) Depts. of Pharm. & Physiol.Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637 and Robarts Res. Inst., Univ. Western Ont., London, ONT

Calcitonin gene-related peptide-like immunoreactive (CGRPir) innervation of the insular visceral sensory cortex (IC) in the rat is thought to originate from the ventroposterior parvocellular (VPpc) visceral relay nucleus in the thalamus. We re-examined this putative projection, using immunohistochemistry for CGRPir combined with retrograde transport of the fluorescent dye, Fluoro-gold (FG).

Only modest numbers of CRGPir fibers were seen in the dysgranular and agranular IC, and fewer still were found in the granular IC. However, the density of CGRPir innervation increased caudally along the rhinal fissure, in the adjacent perirhinal cortex. Following injections of FG into the granular or dysgranular IC, numerous retrogradely labeled neurons were seen in the VPpc, but few if any of these were CRGPir. On the other hand, injections of FG into the perirhinal cortex retrogradely labeled numerous CGRPir neurons in the posterior thalamic complex.

We did, however, observe that the VPpc was heavily invested with CGRPir terminals. Injections of FG into the VPpc retrogradely labeled CGRPir cell bodies in the parabrachial nucleus. Additional CGRPir cell bodies in the parabrachial nucleus were retrogradely labeled by injections of FG into the IC. CCRP may be a neuromodulator in the ascending visceral sensory pathway from the parabrachial nucleus to the VPpc and the IC, but not between the latter structures.

527.30

EFFERENT PROJECTIONS FROM THE INFRALIMBIC CORTEX. K.M. Hurley and C.B. Saper, Dept. of Pharm. & Physiol. Sci., University of Chicago, Chicago, IL 60637.

Electrical stimulation of the infralimbic cortex (ILC) decreases blood pressure and inhibits gastric motility. We examined the efferent projections of ILC using the anterograde tracer PHA-L. Three efferent fiber bundles were observed. (1) A dorsal projection innervated the prelimbic and anterior cingulate cortices, then continued into the dorsal fornix and the dorsal subiculum. (2) Fibers in a lateral projection either extended caudally in the ventromedial portion of the internal capsule or laterally to innervate the dorsal piriform and insular cortices. (3) The largest group of labelled fibers took a ventral trajectory, entering the medial forebrain bundle (MFB) to innervate portions of the basal forebrain, hypothalamus, midline thalamus and ventral tegmental area. At the posterior hypothalamus, some MFB fibers coursed dorsomedially into the periaqueductal grey while others extended dorsolaterally into the central tegmental fields. Both pathways contibuted to terminal labelling in the parabrachial nucleus. Fibers descended through the medial and lateral portions of the medullary tegmentum and innervated the spinal trigeminal nucleus, rostral ventrolateral medulla, nucleus ambiguus and the ventrolateral, medial and commissural portions of the nucleus of the solitary tract. These efferent projections may provide the neuroanatomical substrates mediating the physiological responses observed during electrical stimulation of ILC.

527.31

SINGLE-UNIT ACTIVITY IN THE BED NUCLEUS OF THE STRIA TERMINALIS (BST): RESPONSES TO PERIPHERAL THERMAL AND BARORECEPTIVE INPUTS. W.B. MATHIESON, Q.J. PITTMAN AND W.L. VEALE. Dept. Med. Physiol., University of Calgary, Alberta T2N 4N1.

Arginine vasopressin (AVP), released from nerve terminals in the ventral septal area (VSA), acts as an endogenous antipyretic to control febrile hyperthermia (J. Physiol.387:163-172). A major source of this AVP is from neurons in the BST. To characterize the connectivity of these cells with their targets in the VSA, and the conditions under which they are activated, single-unit extracellular recordings were made from BST neurons in anesthetized rats. Afferent and efferent connections were identified by electrical stimulation of the med. amygdala and VSA. BST neurons received both inhibitory and excitatory inputs from the amygdala and VSA. Efferents to the VSA were identified by stimulus-evoked antidromic spike invasion. The response of BST neurons to changes in scrotal skin temperature or increases in BP was recorded to investigate if previously described thermoresponsive and baroresponsive neurons in the VSA get peripheral receptor input via this BST-VSA pathway. Although some units were sensitive to scrotal temperature or BP, the results suggest that the BST is not a major relay of these inputs to the VSA.

528.1

ANATOMICAL AND MORPHOLOGICAL STUDY OF EXTRAADRENAL CHROMAFFIN TISSUE IN YOUNG RABBITS, PRE- AND POSTSTRESS
J.A. Mascorro* and O.A. Galindez*. (SPON: J.T. Weber). Dept. of Anatomy, Tulane Med. School, New Orleans, LA.

The adrenal medulla possesses abundant catecholamines (CAMs) and peptides. While the role of the latter is not understood, it generally is agreed that CAMs are released from this endocrine gland in response to various stimuli. Abundant chromaffin tissue also exists in extraadrenal sites, but the function of these "paraganglion" cells is totally unknown. In order to determine if this tissue responds to stimulus as does the adrenal medulla, animals were subjected to a hypothermic environment known to affect the CAM-rich cells of the medulla. Tissues were anatomically mapped with potassium dichromate and then processed for fine structural study. Following mild (4 hrs. @4°C, 34°C rectal temp.) or more severe (10 mins. in 0°C ice bath, 15°C rectal temp.) hypothermic stress, medullary CAM granules readily showed changes ranging from partial to total depletion of the dense cores usually associated with CAM storage. Conversely, even the more severe hypothermia failed to affect the paraganglion granules to the same degree, as the granule core always was present. Occasionally, many granules were swollen and vacuolization was evident within the cytoplasm, nevertheless frank depletion was not noticed. Paraganglion cells may not respond to this stimulus, particularly when one considers that they do not receive nerve endings.

528.2

EFFECT OF MATERNAL HYPOXIA ON ENKEPHALIN AND CATECHOLAMINE LEVELS IN RABBIT PUP ADRENAL MEDULLA.
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Maternal Hypoxia (MH) alters pre and postnatal opiate levels in rabbit brainstem (Dev. Neurosci. In Press). We hypothesized that MH would also alter pre and postnatal adrenal opiate levels. 12 pregnant animals were placed in environmental chambers at gestational day E10. Between days E14 and E28, 6 pregnant animals breathed 21% O2(C) and 6 pregnant animals breathed 14% O2(H). On E28, 3C and 3H animals were delivered by hysterotomy. The remaining animals kindled at term. H was discontinued on day E29. Pups were sacrificed on E28 and postnatal days P3, P7 and P21 and adrenal medullae removed for measurement of opiate and catecholamine levels (NE,DA,E). NE, DA, E content/mg protein were unaffected by MH. Opiate results are below (pmol/mg protein ± SD).

AGE:	Native(N)	Enkephalin H:C	Total(T)	Opioid Activity H:C
E-28	24±12	: 14±5	112±52	: 43±16
P-3	23±12	: 37±18	43±21	: 61±37
P-7	33±13	: 52±18	124±71	: 132±42
P-14	74±47	: 75±20	294±148	: 253±102
P-21	28±14	: 61±22	199±128	: 379±171

MH elevated N and T opioid activity in fetal adrenal medullae ($p<0.05$). MH reduced N and T opioid levels at P21 ($p<0.05$) and accelerated the timing of the postnatal enkephalin peak. MH alters the content, processing, maturational patterns of adrenal medullary enkephalins in offspring.

528.3

INTERACTIONS OF STRESS, SALT INTAKE AND EXERCISE ON ADRENAL AMINES IN THE BORDERLINE HYPERTENSIVE RAT (BHR).
E.F. O'Connor, R.H. Cox, S.K. Naylor* and J.E. Lawler. University of Tennessee, Knoxville, TN 37996.

The BHR (the F1 cross of SHR x WKY) has a marked sensitivity to tail shock stress and dietary salt intake. Since the adrenal medulla and its amine neurohormones are thought to play a permissive role in the development of hypertension, we examined adrenal amines (norepinephrine, NE, epinephrine, E, dopamine, DA, and serotonin, 5-HT) in BHRs given normal (N) or high salt (3% NaCl) diets and exposed daily to swimming, tail shock or neither. Our results (MN±SEM) are summarized below.

		NE(ug)	E(ug)	DA(ng)	HT(ng)
CONT	(N)	5.84(0.4)	25.6(1.1)*	219.73(40)	29.55(4)*
	(3%)	5.31(0.3)	19.8(1.4)	216.97(14)@	20.20(2)
SWIM	(N)	7.10(1.0)*@	24.3(1.5)	256.97(17)*	23.04(3)
	(3%)	6.51(0.5)	21.9(0.9)	175.42(9)	22.63(4)
STRS	(N)	5.00(0.3)	21.9(1.2)	155.25(11)@	22.04(3)
	(3%)	5.56(0.3)	22.8(2.1)	170.19(20)	20.47(3)

* indicates p 0.05 for comparisons between conditions
@ indicates p 0.05 for comparisons between diets.

Swimming had the greatest effect, resulting in an apparent increase in adrenal NE and DA. (Supported by HL19680, HL01395 and HL34878)

528.4

INCREASED ANGIOTENSIN II RESPONSES ARE NOT REQUIRED FOR POTENTIATION OF ADRENAL CATECHOLAMINE RESPONSES TO REPEATED HEMORRHAGE IN DOGS.
J.S.Cross*, M.P.Lilly*, E.J.DeMaria*, and D.S.Gann, (SPON: D.Carlson) Dept. of Surgery, Brown University/Rhode Island Hospital, Providence, Rhode Island, 02903

Potentiated adrenal catecholamine(CA) secretory responses have been described to the second of two paired hemorrhages(hem) in dogs. Angiotensin II (A-II) is an adrenal CA secretagogue and may be necessary for normal adrenal CA responses to hem. To study the role of A-II in the CA response to repeated hem, we prepared dogs (n=13) with splenectomy and adrenal vein and femoral artery and vein cannulas. Two days later under pentobarbital anesthesia, dogs were bled either 20%(H1) of measured blood volume (Evans blue dye), or 0% (H0, samples only). Shed blood was reinfused 30 min after H1. 90 min after the start of the experiment, all animals had 20% hem (0%/20%=H0/H02;n=6), (20%/20%=H1/H12;n=7). Adrenal venous and arterial plasma samples and hemodynamic measurements were taken at -5, 2, 4, 6, 8, 10, 15, 20, and 30min after each hem. CA were measured by HPLC and adrenal secretion rates of epinephrine and norepinephrine (E, NE) were calculated. A-II was measured by RIA (n=4 for H02); data were analyzed by ANOVA. Resting E, and NE were not different among groups, but resting A-II was greater before H12. NE response paralleled E.

Horm	Hem	n	Control	Δ at 10min	Δ at 20min	Δ at 30min
Δ E	H02	6	1.4±0.8	29.2± 9.9*	52.9±26.7*	18.1± 12.8*
(ng/min)	H1	7	2.5±0.7	15.3± 7.1*	33.5±14.1*	33.5± 16.2*
	H12 §	7	2.8±1.5	109.0±59.7*†	116.4±68.8*	120.3± 72.6*
Δ A-II	H02	4	103.9±23.7	119.1±28.3*	150.5±48.9*	237.0±121.3*
(pg/ml)	H1	7	80.3±17.6	170.8±23.2*	198.2±36.0*	208.3± 45.3*
	H12	7	168.0±50.8†	189.3±41.3*	156.8±36.5*	181.5± 32.0*

*$p<0.01$ from control †$p<0.05$ from H1. §$p<0.05$: H12 group vs. H1 group.

Hemodynamic responses in the three hem groups were not different. Maximal Δ E correlated with resting A-II ($p<0.0001$), but not with maximal Δ A-II. Thus the larger E responses to H12 were associated with higher resting A-II but not with greater change in A-II. These results could be explained by increased sensitivity of the sympatho-adrenal system to A-II, action of another factor, or a "permissive" role for A-II in CA responses to hem. Supported by NIH Grant # GM27946.

528.5

EFFECTS OF CHRONIC INSULIN TREATMENT ON ADRENAL CATECHOLAMINE SYNTHESIS AND RELEASE IN THE PITHED RAT.
K. Richter*, E. M. Stricker and R. R. Vollmer*. (SPON: R.J. Ertel). Dept. Behavioral Neuroscience, Univ. Pittsburgh, Pittsburgh, PA 15260

Hypoglycemia produced by repeated treatment with insulin results in increased adrenal medullary catecholamine (CA) synthesis as evidenced by an induction of tyrosine hydroxylase (TH). The relation between augmented TH activity and CA release in the adrenal medulla was examined in male Sprague-Dawley rats.

Rats were treated with protamine-zinc insulin (Lilly) at 12 hr intervals for 4 days (4-8 U/day, s.c.), causing a two-fold increase in adrenal TH activity. Adrenal CA release was assessed in pithed rats 16-18 hr after the final dose of insulin when blood glucose concentration and absolute adrenal CA content had returned to normal levels. Electrical stimulation of the thoracolumbar spinal cord (4-32 Hz) resulted in increments in blood pressure and plasma epinephrine and norepinephrine that were equivalent in insulin- and saline-treated rats. Unstimulated values were also equal. Insulin had no effect on clearance of epinephrine from the plasma. Similar results were obtained when the adrenal was field stimulated in situ at 8 Hz for 1 hr.

These findings suggest that the induction of TH that results from insulin-induced hypoglycemia is not associated with a change in the basal or neurally mediated adrenal CA release. (Supported by NIH Grant MH-29670 and NSF BNS-8518035.)

528.6

SECRETED MOLECULES OF DIFFERENT SIZES TAKE DIFFERENT ROUTES FROM THE ADRENAL MEDULLA.
S.W. Carmichael, G.M. Tyce, T.L. Yaksh,* D.T. O'Connor* and S.L. Stoddard. Depts. Anatomy, Physiology, and Neurosurgery, Mayo Clinic/Foundation, Rochester, MN 55905, Dept. Medicine, Univ. California, San Diego, CA 92161, and Dept. Anatomy, Indiana Univ., Fort Wayne, IN 46805.

Following an insulin-induced hypoglycemic insult, patients demonstrate a peak in plasma chromogranin A (CHR A) levels that lags behind the catecholamine (CA) peak by about 90 min (Clinical Res. 35:605A, 1987). From this observation we suggest that molecules of different sizes secreted from the adrenal chromaffin cell gain access to the circulation via different routes. Plasma and lymph were collected from cannulae placed in the left adrenolumbar vein and thoracic duct, respectively, in halothane-anesthetized cats (N=9). Blood glucose was determined in samples taken from the femoral vein. Insulin (1 U/kg; iv) was given after collection of baseline samples; additional samples were taken over 4 hr. In three cats, the adrenal glands were isolated from the circulation after 4 hrs and lymph collected for an additional 90 min. Free CA (MW=153-183) levels in adrenolumbar venous plasma greatly exceeded those in lymph. Neuropeptide Y (MW=4254) was found primarily in plasma, but was also present in lymph. CHR A (MW=48 kDa) was detected in approximately equal amounts in plasma and lymph. After the adrenals were isolated, CHR A levels dropped in lymph. We conclude that larger molecules secreted from the adrenal medulla may enter the circulation via the lymphatic system.

528.7

CHANGES IN ADRENAL BLOOD FLOW DISTRIBUTION DETERMINED BY NON-RADIOACTIVE FLUORESCENT MICROSPHERES M.S. Jasper*, P. McDermott*, D.S. Gann, and W.C. Engeland, Sect. of Neurobiology/Dept. of Surgery, Brown Univ./R.I. Hosp.,Providence, RI 02902

Changes in adrenal medullary and total cortical blood flow after hemorrhage (hem) have been described using radioactive microspheres (mcs). To assess changes in intracortical adrenal blood flow, a method was used based on microscopic detection of non-radioactive mcs. Injection of different colored fluorescent mcs permitted multiple determinations of blood flow. Pentobarbital anesthetized dogs (n=6) were prepared acutely with left ventricular and aortic catheters for injection and collection of 15 µm mcs, respectively. Adrenal denervation was done unilaterally by cutting the thoracic splanchnic nerve. Injections were made prior to and immediately after 18 ml/kg hem (duration 5-7 min.). Dogs were sacrificed with KCl and adrenals were removed, fixed and sectioned at 80 µm. Using fluorescence microscopy, mcs coated with fluorescent dyes were counted in each section. The number of mcs in the capsule (CAP), zona glomerulosa (ZG), inner cortex (IC), and the medulla (MED) was determined. Prior to hem, CAP received 10.2% , ZG received 65.4%, IC received 5.5%, and MED received 18.9% of adrenal blood flow. Denervation did not change this distribution. Following hem, in intact glands, the proportion of flow to the MED increased to 55.7%, while proportions to CAP, ZG, and IC decreased to 5.6, 35.1, and 3.5%, respectively. Distribution of intracortical blood flow did not change. In denervated glands, changes in all zones after hem were reduced. Since sinusoids in the IC are in series with ZG capillaries, the small proportion of mcs in IC may result from trapping of mcs in the ZG. To test this possibility, 5 and 8 µm mcs were injected. The ratio of IC spheres to total adrenal mcs increased inversely with sphere diameter, suggesting that blood flow determination with 15 µm mcs underestimates IC flow. These data show that whereas hem increases medullary blood flow in intact glands, neither hem nor denervation causes changes in distribution of intracortical blood flow. These findings support and extend work suggesting that the distribution of adrenal blood flow is influenced by adrenal innervation. Supported by NIH grants DK38951 and DK26831 .

528.8

INFLUENCE OF CAPTOPRIL ON NEURALLY-EVOKED CHANGES IN ADRENAL CORTICAL AND MEDULLARY SECRETION W.C. Engeland, P. Miller* and D. S. Gann, Sect. of Neurobiology/ Dept. of Surgery, Brown Univ./ R. I. Hosp., Providence, RI 02902

Angiotensin II (AII) stimulates the secretion of cortisol (F) from the adrenal cortex and the secretion of epinephrine (E) and norepinephrine (NE) from the adrenal medulla in dogs. Splanchnic nerve stimulation in dogs results in increased secretion of F, E and NE. To test the possible influence of AII generated by nerve stimulation on adrenal responses, neurally evoked secretion was assessed with or without blockade of AII converting enzyme by captopril. Dogs under pentobarbital anesthesia were hypophysectomized and prepared with an adrenal venous cannula; the thoracic splanchnic nerve was cut and placed in a stimulating electrode. Dogs infused with ACTH (2 and 10 ng/min) or with ACTH and captopril (2 µg/kg/min) underwent nerve stimulation (30V; 0.5 msec pulses) at 1 and 4 Hz. Adrenal venous E and NE were measured by HPLC-EC; F and 11-deoxycortisol (S) by HPLC-UV; and AII by RIA. At both doses of ACTH, nerve stimulation resulted in frequency dependent increases in mean arterial pressure (MAP), adrenal blood flow (ABF), arterial AII and secretion of E and NE; at 10 ng/min ACTH only, nerve stimulation at 1 and 4 Hz increased secretion of F and decreased secretion of S resulting in increases in the F/S ratio. Captopril reduced basal MAP and arterial AII and blocked neurally evoked increases in AII. With captopril nerve stimulation resulted in augmented increases in ABF, in adrenal vascular conductance (ABF/MAP) and in E and NE secretion but resulted in similar changes in F and S secretion relative to dogs without captopril. These data show that adrenal steroid and catecholamine responses to splanchnic nerve stimulation can occur after blockade of increases in arterial AII. These findings suggest that neurally-evoked changes in adrenal steroidogenesis occur independently of an intact renin-AII system. Also, the reduction of the vasoconstrictor activity of AII on the adrenal appears to augment the adrenal secretion of E and NE without increasing secretion of steroids. Supported in part by NIH grants DK38951 (WCE) and GM27946 (DSG).

528.9

PLASMA CORTICOSTERONE RESPONSES TO ELECTRICAL STIMULATION OF THE MEDIAL THALAMUS. J.D. Dunn and I. Calloway*. Dept. of Anat., Sch. Med., Oral Roberts Univ., Tulsa, OK 74171.

To pursue the possibility that the medial thalamus is involved in adrenocortical function, blood samples obtained prior to and following electrical stimulation of female rats were assessed for corticosterone (Cpd B) concentration. Rats were anesthetized with urethane ($1.3g \cdot Kg^{-1}$), tracheotomized, instrumented and positioned in a stereotaxic apparatus. ECG, HR, MAP, and hippocampal EEG were monitored and timed blood samples (0.2ml) were obtained from a tail artery. Blood samples were taken at 0.5 min. prior to and at 5, 10, 15 and 30 min. after initiation of stimulation (monophasic square waves, 100µA, 50HZ, 0.5 msec, 1 sec on/1 sec off for 30 min.). A change in plasma Cpd B was considered different from no change when the average of the 5,10,15 and 30 min. samples deviated by more than 10% from the pre-stimulus level.

Stimulation of all subdivisions of the dorsomedial nucleus produced an increase (+21%) in plasma Cpd B levels. Stimulation of the paratenial nucleus resulted in decreased (-16%) plasma Cpd B levels. In contrast no change in Cpd B levels were observed following sham stimulation or stimulation of other surrounding sites, including the medial habenular nucleus. Collectively these data support the hypothesis that the medial thalamus contains areas differentially involved in adrenocortical function.

528.10

INTRA-INDIVIDUAL VARIABILITY OF BASAL, PEAK, AND DELTA CORTISOL DURING REPEATED ACTH STIMULATION. R.G. Kathol, R.S. Jaeckle, J.F. Lopez*, W. Meller*. Departments of Psychiatry and Internal Medicine, University of Iowa Hospitals, Iowa City, IA 52242.

Six male controls were administered alpha $_{1-24}$ cosyntropin by intravenous bolus at 1600 hour on nine occasions with at least a two day interval between tests. Dosages of 250 ng, 1 ug, and 250 ug were each given on three occasions in random order while plasma samples were obtained at 0, 15, 30, 45, and 60 minutes after injection. Mean baseline, peak, peak time, and delta cortisol levels did not demonstrate carry-over effects from prior testing. The mean intra-individual coefficient of variation (CV) for baseline 1600 hour cortisol levels (mean--11.4 +/- 1.5 ug/dl) was 27%. The mean intra-individual CV for peak cortisol levels was 20%, 12%, and 13% for 250 ng, 1 ug, and 250 ug of ACTH respectively. Peak cortisol levels for the 250 ng, 1 ug, and 250 ug injections were 21.4 ug/dl, 23.3 ug/dl, and 24.9 ug/dl respectively. The mean intra-individual CV for delta cortisol levels was 61%, 38%, and 17% for the three dosages respectively. Peak time occurred at 33 and 35 minutes for the 250 ng and 1 ug injections respectively and at 50 minutes for the 250 ug.

These data suggest that longitudinal evaluation of adrenal responsivity to ACTH within an individual is best performed by measuring the peak response. Use of the submaximal 1 ug dose of ACTH allows assessment of increases as well as decreases in cortisol response without compromising within subject variability. Baseline cortisol levels are less consistent than peak response to ACTH for longitudinal monitoring.

528.11

AMPLITUDE MODULATION OF A BURST-LIKE MODE OF CORTISOL SECRETION GIVES RISE TO THE CIRCADIAN GLUCOCORTICOID RHYTHM IN MAN. JD Veldhuis, A Iranmanesh,* G Lizarralde,* ML Johnson.* Univ of Virginia School of Med, Charlottesville, VA 22908; Dept of Int Med, Veterans Administration Medical Center, Salem, VA 24153.

We examined mechanisms subserving the in vivo circadian rhythm of cortisol in man. Blood samples were drawn at 10 min intervals for 24 hr in each of 6 men to yield well defined episodic cortisol release profiles. A novel multiple-parameter deconvolution model was applied to discriminate number, amplitudes, and durations of all significant underlying cortisol secretory bursts, and simultaneously estimate the endogenous half-life of cortisol disappearance. These experiments disclosed delimited cortisol secretory bursts with a mean interpulse interval of 77±4.0 min. The amplitude of secretory bursts was 0.45±0.044 (mcg/dl/min) and half-duration (duration at half-maximal amplitude) only 16±0.61 min. We calculated an in vivo cortisol disappearance half-time of 73±5.3 min (range 62-97 min) and production rate of 142±14 (mcg/dl/day) (16±1.4 mg/day). Cortisol secretory burst frequency varied 2.2 fold over 24 hr, whereas secretory burst amplitude varied 6.6 fold. We conclude: the nyctohemeral pattern of cortisol variation can be accounted for by a model of amplitude-modulated burst-like cortisol secretion. This eliminates the need to postulate a tonic mode of cortisol secretion in man.

528.12

GLUCOCORTICOID REGULATION IN RESPONSE TO CHRONIC ETHANOL TREATMENT. R.L. Spencer, K. Kimura* and B.S. McEwen, Lab. of Neuroendocrinology, Rockefeller Univ., New York, NY 10021.

The response to an acute dose of ethanol (EtOH) resembles a stress response, characterized by a rapid rise in serum corticosterone (Cort). We examined the effect of chronic (1-3 wks) EtOH treatment on glucocorticoid regulation. In 1 experiment, male Sprague-Dawley rats were given EtOH (2.5-3.0 g/kg i.p., 20% v/v) twice per day (9AM and 4PM) for 18 days. The EtOH treated rats had signs of chronic pituitary-adrenal axis activation, with a significant decrease in body weight and thymus weight, and increase in adrenal/body weight relative to saline injected controls. There was, however, no change in glucocorticoid receptor (GR) Type I or Type II binding levels in the hippocampus (HC). EtOH treated rats also had elevated basal serum Cort levels at 8AM on day 19, but a blunted Cort response to 1 hr of restraint stress relative to control rats. In a second experiment with male Long-Evans rats, the same EtOH treatment paradigm produced a similar pattern of body and tissue weight changes, as well as a significant increase in GR Type I binding in the HC. These results indicate changes of glucocorticoid regulation in response to chronic EtOH treatment. Some of these changes may be adaptive and help to diminish the acute and long-term "stress" response to ethanol. Supported by MH41256 and AA05256.

528.13

VIP BINDING AND STEROIDOGENIC EFFECTS IN THE ADRENAL CORTEX. L.A. Cunningham and M.A. Holzwarth. Department of Cell and Structural Biology, University of Illinois, Urbana, IL 61801.

VIP-immunoreactive nerve fibers innervate the capsule and zona glomerulosa regions of the rat adrenal cortex. Previous studies demonstrated direct VIP stimulation of steroid secretion by rat adrenal capsule-glomerulosa tissue preparations perifused in vitro (Endocrinology 122(5), 1988). The present studies were undertaken to determine: 1) the distribution of adrenal VIP binding sites and 2) whether the steroidogenic effect of VIP is altered by the presence of ACTH or angiotensin II. Using in vitro autoradiography, VIP binding sites were found to be concentrated in the capsule and zona glomerulosa, the regions that receive most extensive VIP innervation. Specificity of ^{125}I-VIP binding in this region was demonstrated; binding of 0.75 nM ^{125}I-VIP was inhibited (65%) in the presence of unlabelled VIP (7.5 μM), was unaffected by ACTH (7.5 μM), and was decreased 20% by 7.5 μM angiotensin II. VIP stimulation of aldosterone, but not corticosterone, was enhanced by the humoral secretaguogue, ACTH;aldosterone secretion stimulated by 10^{-5} M VIP in vitro was enhanced 70-80% by concomitant stimulation with subthreshold concentrations of ACTH (10^{-13} or 10^{-12} M). VIP-stimulated steroid secretion was not affected by the presence of 10^{-10} or 10^{-9} M angiotensin II.

528.14

ONTOGENY AND HANDLING-INDUCED CHANGES IN HIPPOCAMPAL GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS IN THE RAT. V. Viau, A. Sarrieau, S. Sharma, and M.J. Meaney. Douglas Hospital Research Ctr., Dpet. Psychiatry, McGill Univ. Montreal, Canada H4H 1R3.

We report here on the developmental changes in ^{3}H RU 28362 (glucocorticoid receptor) and ^{3}H aldosterone (mineralocorticoid receptor) binding capacity in soluble fractions prepared from hippocampal tissue. ^{3}H RU 28362 bind was low on Day 3 of life (30% of that observed in adults) and increased towards adult values during the second and third weeks of life, a pattern virtually identical to that previously reported for dexamethasone binding. In contrast, ^{3}H aldosterone binding on Day 3 of life was only slightly less than that observed in adults and reached adult values by Day 7 of life. Postnatal handling, which has been shown to increase dexamethasone binding in hippocampus, resulted in a significant increase in ^{3}H RU 28362 binding capacity in hippocampus, but had no effect of ^{3}H aldosterone binding. These results confirm that handling selectively influences the glucocorticoid receptor system in hippocampus. (Supported by MRCC grants to MJM).

528.15

BEHAVIORAL AND NEUROENDOCRINE REGULATION OF MINERALOCORTICOID AND GLUCOCORTICOID ACTION. H. Coirini*, J. Schulkin* and B. McEwen, Lab of Neuroendocrinology, Rockefeller University and Dept. of Anatomy, University of Pennsylvania. (SPON: M. Tordoff)

During states of extracellular fluid and body sodium loss the mineralocorticoids (MIN) are elevated in the body and play a fundamental role in the retention and redistribution of body sodium. The hormone also generates a hunger for salt in the rat. The glucocorticoids (GLU) are to a much less degree elevated during body sodium depletion and are not natriorexigenic in the rat. While both hormones compete for the same receptor sites in several limbic brain regions, and there is a far greater number of GLU than MIN sites, these sites also show preferential binding. A MIN target is the preoptic area and hypothalamus; a GLU target is the hippocampus. In the present study we confirm and extend that MIN induced salt appetite in addition to body sodium depletion induced salt appetite is further elevated when combined with GLU. And we report that corticosterone administration increases the level of aldosterone binding to preoptic-hypothalamic, but not to hippocampal MIN receptors. One hypothesis is that elevating MIN receptors by GLU action in preoptic-hypothalamic tissue contributes to the natriorexigenic actions of the mineralocorticoids.

Supported by NIMH 00678

528.16

CHARACTERIZATION OF ^{3}H-RU 28362 BINDING TO TYPE II GLUCOCORTICOID RECEPTORS IN NEURONAL, LYMPHOID AND PITUITARY TISSUES. M.T. Lowy, Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

Glucocorticoid receptor (GR) abnormalities are present in the lymphocytes of depressed patients. However, it is not known if GR in lymphoid tissues are regulated in a manner similar to neuronal GR. To initially address this issue, type II GR were quantitated in neuronal (frontal cortex: FCX, hippocampus: HPC, and hypothalamus: HYPO), lymphoid (circulating lymphocytes: LYMP, thymus: THY and spleen: SPL) and pituitary (PIT) tissues in adrenal-intact and 1 day adrenalectomized (ADX) rats using the specific type II GR ligand ^{3}H-RU 28362.

^{3}H-RU 28362 binding was detectable in all tissues with the rank order of GR content in ADX rats being THY > FCX=SPL>HPC=PIT > HYPO > LYMP. The rank order of GR content in intact rats was THY > FCX=PIT=SPL > HPC > LYMP > HYPO. Tissues from intact rats generally had fewer GR compared to ADX rats. The dissociation constant (K_d) for the GR was similar (0.20 - 0.40 nM) in all tissues and in intact vs. ADX rats. Results from competition studies will also be presented.

These results document the presence of specific high affinity type II GR in neuronal, lymphoid and pituitary tissues.

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528.17

STEROID-RECEPTOR INTERACTIONS IN THE DIFFERENTIAL DOWN-REGULATION OF TYPE I AND TYPE II ADRENOCORTICOSTEROID RECEPTORS IN MOUSE BRAIN. William G. Luttge and Mary E. Rupp*. Dept. of Neuroscience, Univ. of Florida Coll. of Medicine, Gainesville, FL 32610.

Previous work from our laboratory (Luttge and Rupp, STEROIDS, in press) revealed that adrenalectomy-orfadectomy (ADX) of female and male mice resulted in a dramatic bi-phasic up-regulation of Type I, and to a lesser extent, Type II adrenocorticosteroid receptors in mouse brain. Peak levels in maximal binding capacity were observed at 3-8 days post-surgery, followed by decreases to near intact levels by 16 days post-surgery. ALDO and CORT replacement therapy was found to be equally effective in supressing the increases in the binding capacity of both of these receptors. This equivalency is consistent with the equal affinity of ALDO vs. CORT for Type I receptors, but it is inconsistent with the much lower affinity of ALDO vs. CORT for Type II receptors. In an attempt to explain these data we hypothesized that ALDO may down-regulate Type II receptors via its interactions with Type I receptors which, in turn suppress the expression of the gene coding for Type II receptors. The present study offers the first indirect support for this novel hypothesis.

At 24 and 48 h post-ADX, adult female mice were given a single injection of one of the following: 1) Proplylene glycol, 2) ALDO, 3) CORT, 4) RU26752, 5) RU38486, 6) ALDO + RU26752, 7) ALDO + RU38486, 8) CORT + RU26752, or 9) CORT + RU38486. At 72 h post-ADX, mice were killed, brain cytosol prepared, and Type I ([^{3}H]ALDO + [^{1}H]RU26988 ± [^{1}H]ALDO) and Type II ([^{3}H]DEX ± [^{1}H]DEX) receptor binding capacity determined. Once again, ALDO and CORT were found to be equally potent in suppressing Type I and Type II receptor binding capacity. The antimineralocorticoid RU26752 was found to partially suppress Type I receptors and to have no effect on Type II receptors, whereas the antiglucocorticoid RU38486 had no effect on Type I receptors and a marked down-regulatory effect on Type II receptors. Since RU26752 also inhibited the down-regulatory actions of ALDO and CORT on both Type I and Type II receptors, we speculate that ALDO/CORT-Type I receptor interactions may at least in part mediate the down-regulation of Type II receptors.

(Supported by NIH grant NS-24404.)

528.18

Characterization of Glucocorticoid-Type II Receptors in Brain Cell Cultures. Yun-Chia Chou*, William G. Luttge, and Colin Sumners (SPON: W.A. Friedman). Depts. of Neuroscience & Physiology, Univ. of Florida Coll. of Medicine, Gainesville, FL 32610.

The purpose of this investigation was to study the properties of glucocorticoid-Type II receptors in brain cell cultures. Cytosol was prepared from primary cultures of neurons or glia and incubated with [^{3}H]DEXamethasone at 0°C. Free steroid was removed afterwards by gel filtration on G-25 columns. At a steroid concentration of 20 nM, [^{3}H]DEX-Type II receptor binding in cytosol prepared from both cultures reached equilibrium after 16 h incubation and was stable up to 48 h. The binding was saturable and specific. Scatchard analyses of [^{3}H]DEX binding revealed a single population of high affinity receptors in both cultures. Despite no significant difference in the dissociation constant, glia contained 5-20 times as many receptors as neurons. As neuronal cultures are maintained in medium containing plasma-derived horse serum (PDHS), whereas fetal bovine serum (FBS) is used for glial cultures, the potentially differential effects of these serums on receptor binding was examined. Incubation of glial cultures in FBS was found to significantly increase Type II receptor content when compared to incubations in PDHS. Switching cells from serum-supplemented to serum-free medium prior to cytosol preparation was also studied and found to reduce the association rate of [^{3}H]DEX-Type II receptor complexes while having no effect on the K_d or B_{MAX}.

These results demonstrate the presence of glucocorticoid-Type II receptors in brain cell cultures which are similar to those in brain and other tissues. Since glucocorticoids have profound influences on brain function, the use of neuronal and glial primary cell cultures should be an important model system for further research on the molecular mechanisms of glucocorticoid actions in brain. *(Supported by NIH grants HL-36645 and NS-24404)*

528.19

ACUTE EFFECTS OF CORTICOSTERONE ON RAT HIPPOCAMPAL PERIKARYA, M.M. Miller, E. Antecka,* R. Sapolsky, Department Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada, H3A 1A1; Department of Biology, Stanford University, Stanford, Ca., 94305.

This study was designed to identify effects of corticosterone (cort) on hippocampal neurocytoarchitecture using quantitative electron microscopy. Sections from regions CA3B, CA1, and dentate gyrus were examined in unstressed controls and cort-treated rats (20 mg/kg in sesame oil/day x 3 days); each dose produces cort levels in the upper physiologic range for 20 hrs. The density and surface area of Golgi and the number of Golgi stacks in cort-treated rats increased 25-35% in neurons of these regions ($p<.05$, ANOVA). In addition, the surface area of rough endoplasmic reticulum and numbers of cisternae increased (38-50%) in neuronal perikarya ($p<.001$, $p<.003$). Densities of mitochondria, multivesicular bodies, and lysosomes in perikarya were unaffected. These data suggest that high doses of cort not only increase protein synthesis, but also may alter subsequent processing by the Golgi and they agree with studies of Finch et al. (1987) showing a similar time course of cort induction of an array of hippocampal mRNAs. Supported by NIH HD 19431, NIH AG-06633, and FRSQ.

528.20

ACTIVATION OF SYNAPTIC PLASMA MEMBRANE CALCIUM-ATPASE BY GLUCOCORTICOIDS. P.Y. Sze and Z. Iqbal. Dept. of Pharm., Chicago Medical School, North Chicago, IL 60064

Synaptic plasma membrane (SPM) from rat brain is known to have specific binding sites for glucocorticoids. We have previously shown that binding of corticosterone (CS) to SPM promotes membrane binding of calmodulin (CaM). In this study, we report that exposure of the membrane to CS results in similar increases of the activity of calmodulin-dependent Ca-activated ATPase (CaM-Ca-ATPase; "Ca-pump" ATPase). Purified SPM was prepared from rat brain by sucrose-density gradient centrifugation of osmotically disrupted synaptosomes. The membrane was preincubated with CS (10 nM-1 uM) at 37C for 15 min or 0C for 1 hr. CaM-Ca-ATPase activity was determined in the presence of Ca-CaM and defined as (Ca+Mg)-ATPase activity sensitive to trifluoperazine. The steroid hormone produced a dose-dependent increase in the activity of the membrane enzyme; at 1 uM CS, the enzyme activity was more than doubled. The dose-response relationship parallels the specific binding of the steroid to the membrane. Deoxycorticosterone, which does not bind to SPM and which is ineffective in promoting CaM binding to the membrane, was without an effect on the enzyme activity. These data are in support of our hypothesis that the alteration of membrane binding of CaM by glucocorticoids leads to cascading alterations of membrane functions involving Ca-CaM. Membrane transduction of steroid hormone signals may very well involve Ca-CaM.

MESSENGER RNA REGULATION V

529.1

GENE EXPRESSION CHANGES IN DEVELOPING AND MUTANT CEREBELLAR PURKINJE CELLS. A. Messer, E.L. Wylen, J.A. Plummer-Siegard & M.C. Wilson. Wadsworth Ctr. for Labs. and Res., NYS Dept. of Healt & Sch. of Public Health Sci., SUNY, Albany, N.Y. 12201 & Res. Inst. of Scripps Clinic, La Jolla, CA. 92037.

Branks and Wilson (Mol. Brain Res, 1:1-16;1986) have described a cDNA clone, pMuBr3, which, in adult cerebellum, preferentially hybridizes to Purkinje cell soma in situ. In situ hybridization (^{35}S antisense RNA) studies of developing mouse cerebella show that at postnatal day(P)0 there is no label over the external granule layer (EGL) and heavy label over the developing molecular layer (ML) and Purkinje layer (PL). The P7 pattern is similar, uniformly heavy in the ML, plus light label over the developing internal granule layer (IGL). By P14 there is a significant shift, with the heaviest label over the PL, and much less in the ML. The adult has only scattered label in the IGL and ML, with very heavy label over the PL. The residual, abnormal Purkinje cells in the staggerer mutant show greatly reduced labeling, with somewhat more ML signal than controls. These results are consistent with a developmental shift from dendritic to somatic localization of the mRNA recognized by pMuBr3 in normal Purkinje cells, although the P0 pattern is so diffuse that additional factors must also be involved. Preliminary analysis by Northern blot suggests that total pMuBr3 mRNA increases greatly between P0 and P14, with some discordancy between the two reported transcripts. Studies of additional ages and mutants are in progress. Supported by NS17633 (AM) and NS23038 (MCW).

529.2

INDUCTION OF NOVEL SPECTRIN ISOFORM DURING PERIOD OF SYNAPTOGENESIS. J.C. Noszek*, R.B. Nelson, L.G. Davis and R. Siman (SPON: J. Schwaber). Medical Products Dept., The DuPont Co., Wilmington, DE. 19898.

The actin-binding structural protein brain spectrin (also called fodrin) exists in mammalian central neurons in two isoforms. The two share a similar, if not identical subunit (Mr~240kD, α-spectrin), and have immunologically distinct second subunits (Mr~235kD, β-spectrin and δ-spectrin). The $\alpha\beta$-isoform is present in undetectable levels before post-natal day (PND) 7, and is induced over the next two weeks by more than 20-fold. Here, we have examined the developmental appearance of mRNA encoding β-spectrin. We used a 1kb cDNA selected from a human reticulocyte library (Prchal *et al.*, PNAS *84*, 7468). A portion of the amino acid sequence predicted from the cDNA completely matches the known partial sequence of the erythrocyte β-subunit. The probe labels a single band of about 9.5kb on Northern blots of adult rat brain and two other tissues known to contain the $\alpha\beta$-isoform, cardiac and skeletal muscle. All brain regions examined contain the 9.5kb mRNA, with levels in cerebellum, the most enriched region, exceeding those of thalamus by about 8-fold. Similar to the β-spectrin polypeptide, the mRNA cannot not be detected in cerebral cortex before PND7, and rises until PND 24, an increase of more than 10-fold. In contrast, mRNA for another structural protein, actin, does not appreciably change over the same post-natal period. These results indicate that expression of the $\alpha\beta$-spectrin isoform is controlled primarily by the level of β-subunit mRNA, and suggest that synapse formation could signal the induction of β-spectrin gene expression.

529.3

GENE EXPRESSION IN RAT CEREBELLAR DEVELOPMENT. S-C. Lin* and M.R. Morrison-Bogorad. Depts. of Biochemistry and Neurology, UT Southwestern, Dallas, TX 75235.

We have examined the temporal expression of individual cerebellar mRNAs by plaque differential screening and by Southern analysis of clones from a 20 day rat fetal cerebellar cDNA library cloned in lambda-ZAP. Seven hundred plaques were screened by plaque differential hybridization to cDNA probes for fetal and adult cerebellar mRNAs; 8 plaques were selected for further analysis. Southern hybridization confirmed that four of these contained sequences highly enriched in fetal cerebellum. Northern analysis showed that two mRNAs, 0.7 and 1.8 kb in size, were expressed at high abundance in rat fetal cerebellum and cortex. The mRNAs were present in adult testis, were 30-60 fold reduced in adult cortex, and were not detected in adult cerebellum, liver or kidney. In a parallel series of experiments, cDNA was isolated from 500 individual plaques (pooled into 150 groups) using our modified rapid mini-isolation method. Southern analysis of the restricted DNAs showed that >90% of the inserts were detected by the fetal probe. Thus, this method can be used to screen mRNAs of low abundance. Most of the fetal cerebellar mRNAs were expressed at similar levels in the adult rat cerebellum. 20% were reduced several fold and 2% were increased in development. Five mRNAs were highly enriched in fetal cerebellum. Supported by NIH HD14886 and the Leland Fikes Foundation.

529.4

DEVELOPMENTAL REGULATION OF GAP-43 mRNA.
S. G. McElligott, L. G. Davis, and J. G. McElligott#
Medical Products Dept., E. I. du Pont Company, Wilmington, DE 19898 and #Dept of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

Growth-associated protein (GAP-43/B50/pp46/F1) has been implicated in axonal elongation, regeneration, and synaptic plasticity. This neuron-specific phosphoprotein is expressed at high levels during the first week after birth and decreases to lower levels in the adult brain. With the single cell resolution of in situ hybridization histochemistry, we determined the cellular location of GAP-43 messenger RNA (mRNA) during postnatal development in various brain areas. Regional differences in the ontogenetic pattern of this mRNA expression are observed. The densest hybridization signal is observed in the neocortex, thalamus, ventromedial hypothalamus, hippocampus, and amygdala from birth to postnatal day 7. At a cellular level, the expression in various neuronal cell types changes over the developmental time course. For example, at birth high levels of this mRNA are observed in neurons of the cortical plate and pyramidal layer of the hippocampus whereas in the later emerging granule cells of the dentate gyrus and intermediate zone of the cerebral cortex increased hybridization is observed only after postnatal day 7. Maximum expression of GAP-43 mRNA in these cell types coincides with periods of dendritic and axonal growth and synaptogenesis. The developmentally coordinated expression of GAP-43 mRNA supports a role for this protein in axonal elongation and synaptic growth.

529.5

IDENTIFICATION of mRNA SPECIES RAPIDLY INDUCED BY NGF TREATMENT OF PC12 CELLS. Jeffrey L. Twiss* and Gary E. Landreth (SPON. R. Hadley), Department of Neurology, Medical University of South Carolina, Charleston, SC 29425.

NGF rapidly stimulates the transcription of the protooncogene c-fos; this gene can be superinduced by pretreatment of the cells with protein synthesis inhibitors. In order to identify other NGF-induced mRNA species we constructed a cDNA library in lambda gt10 from PC12 cells treated with NGF for 30 min in the presence of anisomycin. The library contained 2 x 10^5 recombinants and was initially screened using a subtracted probe and rescreened twice by differential hybridization. Approximately 20 positive clones were identified. Ten of these have been further investigated. The mRNA levels were maximally elevated within 30 min (1 clone), 1 hr (5 clones), or 4-10 hrs (4 clones) following NGF treatment. The expression of three clones was NGF specific, while the mRNA levels of the other clones were also elevated by EGF and TPA.

We have independently identified several additional mRNA species whose abundance is decreased following NGF treatment. One such clone, 1N18, is suppressed after 30 min of NGF treatment, but subsequently rises to control levels after 1 h.

529.6

INDUCTION OF HEAT SHOCK (STRESS) GENES IN THE MAMMALIAN BRAIN ANALYZED BY IN SITU HYBRIDIZATION. T.E. Masing and I.R. Brown. Department of Zoology, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada M1C 1A4.

Studies in this laboratory have described striking regional differences in the expression of genes encoding 70 kDa heat shock proteins (hsp70) in the rabbit brain (Sprang and Brown, Molec. Brain Res. 1987, 3: 89-93). This previous work utilized frozen sections (ten microns) for in situ hybridization. To identify the types of neuronal and glial cells which are engaged in the expression of hsp70 genes we have now carried out in situ hybridization utilizing plastic (methacrylate) embedded tissue (sectioned at three microns) which permits increased cellular resolution. The patterns of expression of hsp70 genes have been analyzed in the retina, cerebellum, hippocampus and neocortex of control and hyperthermic rabbits using labeled antisense RNA probes (length 255 nucleotides) transcribed from a mouse hsp70 genomic clone (Perry and Moran, Gene, 1987 51: 225-234). Constitutive expression of an hsp70 gene was noted in several neuronal cell types. Induction of hsp70 mRNA was dramatic in glial cells in fibre tracts throughout the brain and was also apparent in several neuronal cell populations. (Supported by grants from the Medical Research Council of Canada).

529.7

NUCLEOCYTOPLASMIC TRANSPORT OF RECENTLY SYNTHESIZED RNA BY NEURONS IN HIPPOCAMPAL SLICES IS TEMPERATURE SENSITIVE

R. Kleiman*, L. Davis, G.A. Banker[1], and O. Steward (SPON: L. Phillips). Dept. of Neuroscience, and the Neuroscience Program, Univ. of VA, Charlottesville, VA 22908. [1]Dept. of Anatomy, Albany Medical College, Albany, N.Y.,12208.

In the course of studies of dendritic transport of RNA by hippocampal neurons in slices maintained in vitro, we have found that nucleocytoplasmic transport of recently synthesized RNA is virtually eliminated when slices are maintained at room temperature. Hippocampal slices prepared from 21-26 day old Sprague-Dawley rats were incubated with ^{3}H-uridine (.3mCi/ml) for one hour at room temperature (about 27°C) or at 37°C. Following the one hour labeling period, all slices were rinsed in aerated Ringer's solution with an excess of unlabeled uridine (10^{-4}M) and maintained at the same temperature as the incubation solution. Slices were fixed in 10% buffered formalin at 0, 1.5, 3, 4.5, and 6 hours following labeling. All slices were embedded in plastic and 2um sections were cut and prepared for autoradiography. At the end of the one hour pulse at 37°C, the label was localized exclusively over the nucleus. Autoradiographs of slices maintained at 37°C show that over time, the distribution of silver grains expanded to cover the entire cell body, although the nuclear labeling remains relatively higher. At room temperature the distribution of label at the end of the pulse (0 hours) was comparable to the slices maintained at 37°. However at longer intervals, these cells exhibited no increase in cytoplasmic labeling, indicating that the lower temperature inhibited nucleocytoplasmic transport of recently synthesized RNA. Electrophysiological studies of slices maintained at room temperature reveal that the slices are viable, and fully capable of synaptic transmission. Thus, the inhibition of nucleocytoplasmic transport of RNA provides an opportunity to evaluate neuron function when no new mRNA molecules can reach the cytoplasm. Supported by NIH NS23094 to GB and OS.

529.8

MOLECULAR, CELLULAR AND GENETIC ANALYSIS OF A NOVEL RAT BRAIN-SPECIFIC PROTEIN 1B1075. H.P. Ottiger* and J.G. Sutcliffe Research Institute of Scripps Clinic, La Jolla, CA 92037

The application of molecular genetics to the study of mRNAs expressed in the brain is essential in order to understand brain function and development. In a study that was undertaken to identify recombinant cDNA clones of mRNAs that code for proteins that are unique to or preferentially expressed in the rat brain we isolated and characterized 1B1075. In northern blot experiments 1B1075 cDNA clones detect two moderately abundant (0.05% of polyA+ RNA) brain mRNAs (2.1 kb 1.9 kb), expressed at comparable levels by different regions of the brain and also by pituitary, but not detectable in any other tissue. Developmental onset occurs earlier than embryonic day 16 and peaks at postnatal days 15-20. The pattern of expression has been analyzed by in-situ hybridization and immunocytochemistry. Hybridization was observed in cellullar layers in the frontal and parietal cortex in granule cells of the dentate gyrus and in Purkinje cells in the cerebellum. Antisera directed against synthetic peptides verify the cellular distribution and reveal the protein to be partially distributed in the fibers that stem from these cells. Analysis of the primary structure deduced from the translation of the DNA sequence of a full length cDNA clone predicts a novel, acidic protein containing 533 residues, with an apparent secretion signal, several potential dibasic cleavage sites and a remarkable string of repeated elements. Chromosomal linkage analysis maps the mouse analogue of 1B1075 near the dilute-short ear (d-se) region of chromosome 9. A mouse strain deleted for the se locus is also deleted for the 1B1075 gene. The mice are viable and don't show an overt phenotype. We conclude that there may be redundancy at the molecular level in the mammalian central nervous system.

529.9

DISTRIBUTION OF mRNA FOR THE A1 ISOFORM OF NaKATPase AND ^{3}H-OUABAIN BINDING IN HUMAN HIPPOCAMPUS. M.L. BRINES*, A. GREENE*, D.D. SPENCER, E.J. BENZ*, AND R.J. ROBBINS, Yale School of Medicine, New Haven, CT 06510

Na-KATPase (NKA) is an ubiquitous enzyme which regulates cell membrane potential and volume. Three isoforms exist, the products of separate genes, which appear to have distinctive biophysical properties and anatomic distributions. We used human hippocampus to compare the distribution of mRNA for the A1 isoform of NKA, which occurs widely in brain and ion transporting epithelia, to that of the mature enzyme, as assessed by ^{3}H-ouabain autoradiography. By in situ hybridization histochemistry performed on surgical specimens of hippocampi, we found that A1 mRNA was present at highest density over the cell bodies of the dentate granule cells. Neurons in the CA fields, subiculum and deep layers of entorhinal cortex also possessed high levels of mRNA for A1. Binding was diffuse and of low intensity in the outer 2/3 and absent in the inner 1/3 of the dentate moleculare layer, as well as in layer I of entorhinal cortex. Specific ouabain binding was more widely and evenly distributed over the same regions labelled for mRNA, and extended into layer I of cortex. Regional density in ouabain binding was much less marked than for mRNA. These data suggest that neurons of the hippocampus, especially dentate granule cells, have a high turnover of NKA compared to surrounding glia, presumably related to their electrical activity.

529.10

LOCALIZATION OF NA,K-ATPASE α-SUBUNIT mRNA AND POLYPEPTIDE IN MOUSE KIDNEY, CEREBELLUM, AND RETINA. V.C. Hieber, G.J. Siegel, T.J. Desmond, J. Liu-Hwa, and S.A. Ernst. University of Michigan, School of Medicine, Ann Arbor, MI 48109

A clone encoding mouse brain Na,K-ATPase α-subunit was isolated from a mouse brain lambda gt11 cDNA library using antisera to bovine and mouse brain α (combined α1 and α2) subunit. The sequence of this clone was found to be most homologous to that of the rat brain α1, rather than the α2 or α3 isoforms. A [^{32}P]RNA antisense probe derived from the cDNA insert, but not the sense strand, hybridized strongly with brain and kidney RNA on Northern blots (single band, 4.5 kb) and with sections of kidney and retina. Specificity for α-subunit mRNA was further indicated by comparing in situ hybridization of [^{35}S]-riboprobe with immunostaining of α-subunit. In kidney, mRNA and α-subunit showed similar differential tubular distributions (distal > proximal >> thin segments). In retina, inner segments of photoreceptors, the inner nuclear layer, and ganglion cell soma were strongly reactive with both probes. α-subunit, but not mRNA, was prominently expressed in plexiform layers and optic fibers. In cerebellar cortex, α-subunit and mRNA were localized in basket and granule cell soma. Basket regions and glomeruli, which contain synaptic input from these cells, were strongly reactive for the α-subunit. Although Purkinje cell soma expressed abundant message, the somal membrane and emerging dendrite exhibited little, if any, polypeptide, suggesting transport of α-subunit to more distant sites. These results suggest that co-localization of polypeptide and mRNA will be useful in mapping expression and developmental regulation of Na,K-ATPase at the cellular level.

Supported by NIH grants NS15935 and 5P60DK20572.

529.11

The role of neuron-glial interactions in the regulation of expression of glutamine synthetase (GS). K. Mearow, J. Mill* and E. Freese. LMB, NINCDS, NIH, Bethesda, MD. 20892

GS catalyzes the formation of glutamine from glutamate and NH_3^+. In the CNS, GS is predominantly localized in astrocytes in vivo. It is possible that interactions between neurons and glia might lead to the repression of neuronal GS expression in vivo; such interactions have been suggested as important in induction of glial GS expression.

We have modelled possible interactions using culture systems including primary rat astrocytes and neurons, C6 glioma, 6-3 astrocytoma and PC12 cells. GS mRNA expression under the various experimental manipulations was examined by Northern analysis using our rat brain GS cDNA as probe.

Primary astrocyte cultures expressed more GS than the neurons or the various cell lines. Coculture of primary astrocytes with cerebellar neurons resulted in a 50% induction of GS mRNA; however, coculture of these neurons with C6 or 6-3 cells showed no such induction. When C6 cells or primary astrocytes cocultured with NGF-differentiated PC12 cells were compared to cocultures with naive PC12 cells, a 2-4X decrease in GS expression was observed. A similar decrease in GS mRNA was seen when granule neurons were cocultured with astrocytoma cells. These results are consistent with glial cells playing a role in regulating neuronal GS expression.

529.12

INSULIN AND IGF-I REGULATE GLUCOSE TRANSPORTER mRNA LEVELS IN PRIMARY CULTURES OF RAT NEURONAL AND GLIAL CELLS. H. Werner*, M. Adamo*, M. Raizada, C.T. Roberts, Jr.*, I. Simpson*, and D. LeRoith. Diabetes Branch and MCNEB, NIDDK, NIH, Bethesda, MD 20892, and Dept. of Physiology, University of Florida, Gainesville, FL 32610.

The study of the potential modulation of the brain glucose transporter (GT) by insulin and insulin-like growth factor I (IGF-I) has been difficult due to the presence of the blood/brain barrier. We have, therefore, investigated the regulatory role of these factors in the expression of a brain GT gene by using primary cultures of neuronal and glial cells prepared from one-day-old rat brains.

Incubation of neuronal cultures with insulin induced a dose-dependent increase in the steady-state level of GT-specific mRNA, as measured by hybridization of a ^{32}P-labeled rat brain GT cDNA probe to Northern blots. The GT mRNA hybridized as a single band of ~ 2.9 kb. Insulin (100 ng/ml) increased GT mRNA two-fold as compared to controls, with maximal expression achieved after 1 to 2 h. IGF-I was more potent than insulin in increasing GT mRNA levels; treatment with 100 ng/ml IGF-I resulted in a 4.5-fold increase in GT mRNA. In cultured glial cells, insulin similarly increased GT mRNA in a time- and dose-dependent manner. These results suggest that insulin-like peptides can modulate the expression of a brain GT gene, and that these peptides may play a physiological role in the energy metabolism of this organ.

529.13

GENE EXPRESSION IN RAT BRAIN ASTROCYTES. Douglas L. Feinstein and Robert J. Milner. Research Institute of Scripps Clinic, La Jolla, CA 92037

Astrocytes are the predominant cell type in the mammalian brain. However, their function and developmental pathways are poorly defined. We are interested in characterizing the molecules involved in the processes of normal astrocyte differentiation and function, as well as astrocytic components whose expression may change in various pathological states. RNA was isolated from primary cultures of rat brain astrocytes, either untreated or after 4 days of treatment with dibutyryl cyclic AMP (dbcAMP). Northern blot analyses of these RNA samples with oligodendrocyte specific probes (PLP, 1B236/MAG) indicate little if any contamination with oligodendrocytes, while hybridization with neuron-specific enolase showed lack of neuronal contamination. cDNA libraries were constructed in Lambda-Gem4 and in the expression vector Lambda-Zap. These libraries are being screened with DNA probes and with antibodies, for selection of both known as well as novel astrocyte-specific molecules. cDNA clones for rat GFAP mRNA have been isolated, and its DNA sequence will be determined and compared to the mouse sequence.

To begin to evaluate changes in astrocyte function due to dbcAMP treatment (which may reflect the reactive gliotic state) we have screened the astrocyte libraries with a cDNA clone of bovine transducin to isolate clones for G-proteins present in astrocytes. Screening of approx. 100,000 primary plaques yielded a single positive clone, which corresponded to an alternatively spliced from of Gs_α. This clone was used to rescreen the 2 libraries, and an additional 25 clones were isolated. Characterization of these clones will allow us to determine the diversity of G-proteins present in astrocytes, and if any forms are preferentially induced by dbcAMP treatment.

529.14

GLIAL FIBRILLARY ACIDIC PROTEIN mRNA IS DECREASED IN RAT BRAIN REGIONS IN RESPONSE TO ACUTE AND CHRONIC CORTICOSTERONE TREATMENT N. R. Nichols, J. N. Masters, D. S. Moradzadeh* and C. E. Finch. Depts. of Biological Sciences and Gerontology, Univ. of Southern California, Los Angeles, CA 90089-0191.

Glial fibrillary acidic protein (GFAP) is a marker of reactive gliosis and is increased several-fold in brain injury, aging, and neurodegenerative disease. In contrast, glucocorticoids, which potentiate hippocampal neuronal damage (Sapolsky et al., Endo. Rev. 7:284, 1986), also decrease GFAP in rat brain (O'Callaghan et al., Neurosci. Abs. 13:1366, 1987). In this study, corticosterone (CORT; 10 mg/day sc in oil) was administered to adrenalectomized (ADX) or intact Fischer 344 male rats for 8 h or up to 3 mo and levels of GFAP RNA in rat brain regions were measured by RNA blot hybridization using a [32]P-cRNA probe to the coding sequence of mouse GFAP. GFAP mRNA was decreased 2- to 5-fold in hippocampus, cortex, hypothalamus and cerebellum in response to in vivo CORT, but did not change in primary cultures of astrocytes in response to hydrocortisone (1 ug/ml). The magnitude of change was similar in hippocampus and cortex for acute and chronic CORT treatment. In separate experiments, we have cloned cDNAs from a rat hippocampal library by differential hybridization to poly A-containing RNA isolated from ADX or ADX + CORT treated rats. Two of these show decreased RNA abundance in response to CORT and cross-hybridize with a mouse GFAP probe; sequence analysis will determine if these are clones for rat GFAP. The finding of a CORT-mediated decrease in GFAP gene activity is intriguing in light of the plethora of inhibitory CORT actions which prevent overshooting of natural defense mechanisms (Munck et al., Endo. Rev. 5:25, 1984) and the possible role of CORT in altered astroglial function which may lead to a potentiation of CNS damage. Supported by John D. and Catherine T. MacArthur Foundation Research Program on Successful Aging, ONR Grant N00014-85-K-0070 and Brookdale Foundation (NRN).

529.15

INDUCTION OF TIS GENES IN RAT ASTROCYTES BY HORMONES AND GROWTH FACTORS. A. T. Arenander, H. R. Herschman* and J. de Vellis. LBES, Brain Research Institute and Departments of Anatomy and Biological Chemistry, UCLA, LA 90024.

The proliferation and differentiation of astrocytes are regulated by a wide spectrum of hormones and growth factors. In order to study the early nuclear events which accompany the induction of glial biochemical and morphological phenotypic change, the expression of a number of TIS genes (transiently-induced sequences) were examined in secondary cultures of rat neocortical astrocytes treated with mitogens or stellation agents. The TIS family of genes, which include c-fos, are characterized by their rapid and transient expression by various agents in murine Swiss 3T3 cells (Lim etal., Oncogene 1:263, 1987) and in rat PC12 cells (Kujubu etal., Oncogene 1:257, 1987). These experiments examined seven distinct TIS clones, including c-fos (TIS 28), from a cDNA library constructed using mRNA induced in murine Swiss 3T3 cells exposed three hrs to the mitogen/promoter agent tetradecanoyl phorbol acetate (TPA) in the presence of cycloheximide. Expression of TIS gene mRNA in astrocytes was studied by Northern blot analysis of total cell RNA. All the members of the TIS gene family are rapidly and transiently induced by TPA and a variety of other mitogens such as EGF, FGF and the ganglioside GM1. Marked interaction between these mitogens was observed. Interaction was also seen in TIS gene induction by insulin, triiodothyronine and hydrocortisone. TIS genes are also induced by the stellation agents dbcAMP and forskolin. It is interesting to note that one of the TIS genes, c-fos, which is observed to be induced only in neuronal cells in vivo, is markedly induced in astrocytes in vitro.

These results indicate that TIS genes are responsive in astrocytes to a wide variety of agents thought to play important roles in brain growth, differentiation and function. TIS genes may be involved in the early events of intracellular signal transduction.

529.16

REGULATION OF SCHWANN CELL GENE EXPRESSION BY CYCLIC AMP. T. Behrman*, K. Mokuno*, D. Pleasure and J. Kamholz*. Dept. of Neurology, Univ. of Pa. Sch. of Med., Phila., PA 19104

Schwann cell expression of the major peripheral nervous system myelin proteins PO and myelin basic protein (MBP) has been shown to be dependent upon axonal contact, while expression of the Nerve Growth Factor Receptor (NGFR) is activated by withdrawal of axons. These effects are thought to be mediated by cAMP both in vivo and in vitro. We have investigated the steady state messenger RNA (mRNA) levels of NGFR, PO, MBP and proteolipid protein (PLP) in Schwann cells cultured in the absence of axons and after induction in 1 mM dibutyryl cAMP. Northern blots of total Schwann cell RNA demonstrate a decrease in NGFR mRNA and a marked increase in both PO and MBP mRNAs after cAMP treatment. No change in the steady state level of PLP message was found, although cAMP induced a dramatic increase in the number of Schwann cells expressing PLP as determined by immunofluorescence microscopy. These results demonstrate the cAMP treatment of Schwann cells mimics axonal contact, and that this effect is reflected in changes in the steady state levels of mRNA for PO, MBP and NGFR. The cAMP mediated increase in PLP expression, however, must occur by some other mechanism. Cyclic AMP thus produces at least three separate effects on Schwann gene expression: an increase in PO and MBP expression and a decrease in NGFR expression at the level of transcription, and an increase in PLP expression at a posttranscriptional level.

529.17

9A6 mRNA, A BRAIN mRNA REGULATED BY THYROID HORMONE ACROSS SPECIES AND TISSUES, [2]S.A. Stein and [2]C. Craft, Depts. of [1]Neurol. and [2]Psychiat., Univ. of Tx. Southwestern Med. Cent., Dallas, Tx. 75235

The gene expression of the adult rat and mouse brain is sensitive to alteration in thyroid hormone levels. 3-5% of the total mRNAs of adult rat brain may be altered by change in thyroid hormone from the euthyroid(ET) to hyperthyroid(HT) state(Stein, 1988, In Press). One of these mRNAs, 9A6(Stein, Soc. Neurosci. Abst., 12:367,1986), was abundant in adult and neonatal rat and mouse liver and brain. In comparing the abundance of this mRNA in ET(vehicle injected) and HT(vehicle + T4 injected) adult rats and mice, 9A6 mRNA is, in the transition from the ET to HT states: 1)Increased in rat liver and total brain (TB); 2) Decreased in mouse TB; 3)Increased in mouse cerebral cortex(CC); and 4)Unchanged in mouse liver on northern gel hybridization(NGH). This demonstrated that thyroid hormone(TH) responsiveness may be related to similar tissue or gene elements across species and across different tissues within a specie. However, the direction of the abundance change in the ET to HT transition in TB and CC may be controlled by other undefined factors. To start to define the tissue and specific gene factors that might be involved, we utilized DNA sequence analysis of the 9A6 cDNA and other TH regulated cDNAs as well as in situ hybridization(ISH) to adult rat and mouse brain. The 9A6 cDNA was sequenced in double stranded form by modified dideoxy sequencing using Sequenase(U.S. Biochemical). Because of secondary structure, the purified 9A6 insert was subcloned into M13 and resequenced by standard dideoxy sequencing.The 350 bp insert was a unique mRNA based on the sequence analysis. The initial search of the Gene Bank revealed no similarities with any known protein. On NGH, 9A6 mRNA was more more abundant in CC and cerebellum than in thalamus or brainstem. ISH of this mRNA to adult ET and HT rodent brain will be reported (Supported by NIMH #43017 and UCP #377-87).

529.18

CHARACTERIZATION OF mRNA COMMON TO NEURONS DESTROYED BY THE SELECTIVE NEUROTOXICANT TRIMETHYLTIN. J.K. Krady,*G.A. Oyler,*and M.L. Billingsley. (SPON: J.S. Long) Hershey Medical Center, Penn State Univ. Herhsey, PA, 17033

Trimethyltin (TMT) destroys selected populations of neurons which have no apparent neurochemical or anatomic linkage. Sensitive neurons may share common gene products which cause TMT toxicity. We have used avidin/biotin subtractive hybridization to generate a library of cDNAs enriched for TMT-sensitive mRNAs. cDNAs were isolated, labelled, and used to probe slot and Northern blots of brain poly(A+) mRNA from TMT and saline-treated rats. Two clones hybridized only to brain mRNA from saline-treated rats suggesting that these mRNAs were selectively lost from TMT-sensitive neurons. Hybridization studies suggest that TMT-sensitive mRNAs are rare (>0.01%). We have used sequences from the cDNAs to make oligonucleotides for probing human and rat cDNA libraries and are pursuing in situ hybridization studies in order to co-localize TMT-sensitive mRNAs in neurons destroyed by TMT. These experiments represent a useful approach for the isolation of toxicant-specific gene products. Supported By EPA-CR-813637.

529.19

S100βExpression is Regulated by Multiple Signal Transduction Pathways. Dana C. Hilt* and Douglas Kligman#. Lab of Biochemical Genetics*, NHLBI and Lab of Molecular Biology#, NINCDS, NIH, Bethesda, Md. 20892.

S100β is a small, Ca^{2+} modulated protein abundant in nervous system glial cells. Recently, neurite extension activity has been ascribed to the disulfide bonded dimeric form of S100β. In order to study the regulation of S100β expression we have obtained and characterized a near full-length cDNA clone from a rat brain cDNA library. This probe was used to examine the regulation of S100β mRNA levels in C6 glioma cells by a variety of pharmacological agents. Forskolin (10uM) significantly elevated the steady state S100β mRNA levels at 48 hours. There is a less marked increase in the mRNA levels with 1uM dexamethasone. However, when both agents are present, the S100β mRNA level is significantly decreased from that seen with forskolin alone. These findings suggest a negative cooperativity, with glucocorticoids inhibiting the cAMP-dependent increase in S100β mRNA. These results are corroborateded at the protein level by immunological methods. In particular, these results suggest that the S100β gene contains both the glucocorticoid regulatory element (GRE) and the cAMP-dependent regulatory element (CRE). Treatment of C6 cells for 48 hours with 10nM PdBu causes a marked suppression in S100β mRNA levels. This type of phorbol ester-dependent response is similar to that seen in other cell types for c-myc and c-fos expression. This result suggests that S100β may have activities related to cell cycle, growth or regulation of differentiation. These results indicate that the S100β gene is under complex regulation and implies that S100β expression is tightly regulated by a variety of extracellular signals.

529.20

LIGHT/DARK CYCLING OF S-ANTIGEN (48-KDa PROTEIN) mRNAs IN RAT RETINAS AND PINEAL GLANDS. C.M. CRAFT. Lab. of Molecular Neuroendocrinology, Dept. of Psychiatry, University of Texas Southwestern Medical Center, and VA-Medical Center, Dallas, TX 75216

During the 24 hour (circadian) period, normal physiological fluctuations occur in visual and signal transduction. These rhythmic circadian changes of neurotransmitters, receptors and second messengers regulate numerous pineal/retinal specific proteins. To extend these studies to the molecular level, complementary DNAs (cDNAs) encoding pineal/retinal tissue specific proteins are being isolated and characterized. Retinal S-antigen (S-Ag) is involved in the down regulation of photolyzed, phosphorylated rhodopsin; however, pineal S-Ag's function is not known. The cDNA for bovine retinal S-Ag (Shinohara et al., 1987, PNAS, 84:6975) was used to examine the 24 hour regulation of its mRNA. Male rats were kept in a light:dark cycle (L:D) (12:12) and killed throughout the cycle. Pineal/ retinal catecholamines and indoleamines were determined by HPLC to verify L:D levels obtained in previous experiments (Craft et al., 1984, Neuroendocrinology, 38:193). Tissue (pineal, pituitary, retina, brain and liver) from each animal (10/group) was used for protein and mRNA analysis. The labelled cDNA probe encoding S-Ag was hybridized with 10 ug of total RNA transferred to Zetaprobe from each time point. The 1.7 kilobase mRNA for S-Ag exhibited L:D fluctuations with peak levels exhibited after 5, 7, and 11 hours of lights off in retina and pineal. No message was detected in any other tissue. The same blots were reprobed with an antisense 18S ribosomal probe to standardize the amount of RNA per lane.

Total RNA was extracted from 150 pineals which had the highest S-Ag mRNA detected. PolyA^+ RNA from pineals (5 ug) was used to synthesize cDNAs and to construct a Lambda ZAP library (Stratagene); $5x10^6$ recombinants were obtained. The cDNAs encoding rat pineal S-Ag and other specific proteins are presently being isolated. (Grant support from Scottish Rite Schizophrenia Foundation).

529.21

INDUCTION OF ASTROCYTE-SPECIFIC mRNAs GFAP AND B-S100 DURING SEIZURE ACTIVITY. C.F. Landry, G.O. Ivy, N.W. Milgram and I.R. Brown. Departments of Zoology and Psychology, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada M1C 1A4

Using immunohistochemical techniques, we have shown that patterns of astrocyte hypertrophy (AH) can be used to trace neural systems which undergo abnormally heightened levels of electrical activity (Ivy and Milgram, Soc. Neurosci. Abstr. 1987, 13: 1265 and this meeting). Using in situ hybridization histochemistry, we now delineate the brain distribution and time course of production of mRNAs for the astrocyte-specific markers GFAP and B-S100 following kainic acid (KA)-induced seizure activity. To this end, rats were injected systemically with KA. Both seizure time and survival time were varied. High GFAP and B-S100 mRNA levels were observed within three hours of seizure onset in specific regions of hippocampus, thalamus, neo- and pyriform cortex, endopyriform nucleus and amygdala. Signals persisted in these regions for at least four months, but at decreased intensity. The distribution of GFAP and B-S100 mRNA mimicked both GFAP immunohistochemistry and the pattern of neuronal damage however the mRNA patterns appear prior to evidence of neuronal damage. Thus, induction of astrocyte-specific mRNA species is an early and sensitive indicator of selective neuronal vulnerability caused by seizure activity. (Supported by grants from NSERC.)

529.22

LAMININ SYNTHESIS IN CULTURED MAMMALIAN ASTROCYTES. Jerome R. Wujek, Robert H. Lipsky*and Ernst Freese. Laboratory of Molecular Biology, NINCDS, NIH, Bethesda, MD 20892.

The control of laminin mRNA and protein synthesis was studied in cultured neonatal rat cerebral cortical astrocytes. Total RNA was extracted from astrocytes grown for 1-10 weeks in DMEM plus 10% fetal calf serum (FCS), then electrophoresed, blotted and hybridized with cDNA probes to the B1 or B2 laminin genes. Results indicated that mRNA for the B2 laminin chain increased in the first 2 weeks in culture and decreased in the next two weeks. In contrast, the B1 chain mRNA was found only after 10 weeks in culture. Thus, synthesis of laminin mRNA for the different laminin protein chains appears to be regulated independently. Immunocytochemistry and ELISA indicated a 10-fold greater laminin expression by astrocytes cultured in serum-free (SF) medium than by astrocytes in serum-supplemented (10% FCS) medium. Addition of transforming growth factor beta increased laminin deposition by astrocytes only in SF medium. Conversely, retinoic acid plus cyclic AMP, when added to astrocytes in 10% FCS medium, produced a 3-fold increase in laminin. Such conditions can be used as a model system for the study of laminin synthesis by astrocytes.

530.1

VARIOUS EFFECTS OF α-BUNGAROTOXIN AND PERHYDROHISTRIONICOTOXIN ON POST-TETANIC POTENTIATION OF MOUSE DIAPHRAGM TWITCH TENSION. M.C.TSAI and M.L.CHEN*. Pharmacol. Inst. College of Med., National Taiwan Univ., Taipei, Taiwan, R.O.C.

Effects of α-bungarotoxin (α-BuTX) and perhydrohistrionicotoxin (H_{12}-HTX) on the post-tetanic potentiation (PTP) of twitch tension were studied on the isolated mouse diaphragm preparation. In normal physiological solution, maximal PTP was obtained after 100 Hz tetanic stimulation for 5 sec. In preparations partially blocked by α-BuTX, PTP was less than in control preparations if tetanically stimulated at 100-200 Hz for 5-10 sec. The relationship between frequency,length of tetanic stimulation and PTP revealed that PTP was independent of the duration and frequency of tetanic stimulation. H_{12}-HTX induced post-tetanic depression of twitch tension. The relationship between frequency,length of tetanic stimulation and PTP revealed that maximal depression effect was observed after 100 Hz of tetanic stimulation for 20 sec. It is suggested that the voltage and time dependent effect of H_{12}-HTX on the endplate channel may contribute to the differences in modes of action of both toxins on the PTP of the twitch tension.

530.2

STRUCTURE-ACTIVITY RELATIONSHIPS OF ANATOXIN ANALOGS AT THE NICOTINIC ACETYLCHOLINE RECEPTOR (AChR). KL Swanson[1], RS Aronstam[2], H Rapoport[3]*, FJ Sardina[3]*, EX Albuquerque[1]. [1]Dept Pharm Exp Ther, Univ MD Sch Med, Baltimore, MD 21201, [2]Dept Pharm Toxicol, Med Coll GA, Augusta, GA 30912, [3]Dept Chem, Univ CA, Berkeley, CA 94720.

(+)Anatoxin-a (ANTX) is a stereospecific, secondary amine which has selective actions on peripheral and central AChR's (*Mol Pharmacol* 29: 250, 1986; *FEBS Lett* 222: 63, 1988). Synthetic analogs of ANTX were used to reveal the structural characteristics of agonists, competitive antagonists and noncompetitive antagonists. AChR-ligand binding was performed on *Torpedo* electroplaque membranes. Muscles from *Rana pipiens* were used for *in vitro* twitch and contracture assays. Data for agonist binding (IC_{50} for α-bungarotoxin (αBGT)) and agonist efficacy (perhydrohistrionicotoxin (HTX) binding or rectus abdominis contracture) were in general agreement. The order of agonist potency was ANTX > dihydroANTX > ANTX methylester > ANTX dimethylamide, with these compounds encompassing a 1000-fold range. N,N-dimethyl ANTX also stimulated HTX binding and weakly inhibited αBGT binding, but did not elicit muscle contractures. Several of the analogs exhibited antagonist effects. The most potent inhibitor of HTX binding was N-methyl-R-anatoxinol, although it is a weak blocker of indirect muscle twitch (sartorius muscle-sciatic nerve preparation). N-methyl and N,N-dimethyl acetoxy ANTX analogs, and N,N-dimethylANTX were antagonists at high μM concentrations. Supported by NIH Grant NS 25296.

530.3

N,N-DIMETHYLANATOXIN: AN ELECTROPHYSIOLOGICAL ANALYSIS. A.C.S. Costa*[1], Y. Aracava*[1,2], H. Rapoport*[3] and E.X. Albuquerque[1,2]. (Spon: D. Weinreich) [1]Lab. Mol. Pharmacol. II, Fed. Univ. Rio de Janeiro, Brazil; [2]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med. Baltimore, MD 21201; [3]Dept. Chem., Univ. California, Berkeley, CA 94720.

(+)-Anatoxin-a, an exotoxin produced by the blue-green algae Anabaena flos-aquae, is a selective nicotinic agonist with high stereospecificity and potency superior to the neurotransmitter on both central and peripheral nAChRs. We analyzed the actions of the synthetic dimethyl analog of (+)-anatoxin-a on single channel currents recorded from isolated fibers of interosseal muscles of the adult Rana pipiens. The two additional methyl groups attached to the nitrogen atom produced a major reduction (1000-fold) of the agonist potency and gain of channel blocking properties. N,N-dimethylanatoxin (20-100 μM) induced a low-frequency, burst activity and a concentration-dependent shortening of the open times and of the burst length as well, suggesting activation and blockade of open ion channels. The blocking action was further analyzed on the currents activated by ACh (0.4 μM) in the presence of the (+)-anatoxin-a analog (1-100 μM). Bursts with increased flickering and a concentration- and voltage-dependent shortening of the open times were observed in accordance with the predictions of the sequential model of open channel blockade. At high concentrations (>50 μM), however, burst length was shortened, indicating an additional blocking pathway. Support: NIH Grant NS25296, CNPq and CAPES-Brazil.

530.4

A QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF NICOTINIC RECEPTOR BINDING FOLLOWING CHRONIC NICOTINE INFUSION. J.R. Pauly, J.A. Stitzel*, M.J. Marks* and A.C. Collins. Univ. of Colorado, Boulder, CO 80309.

Quantitative autoradiographic procedures were used to examine the effects of chronic nicotine infusion on the number of CNS cholinergic receptors. Female DBA mice were implanted with jugular cannulae and infused with nicotine (2.0 mg/kg/hr) or saline for ten days. Nicotinic receptors were labeled with L-[^{3}H]-nicotine and alpha-[^{125}I]-bungarotoxin (BTX); [^{3}H]-quinuclidinyl benzilate (QNB) was used to measure muscarinic cholinergic receptors. Chronic nicotine infusions resulted in site-specific increases in the number of nicotinic cholinergic receptors. For [^{3}H]-nicotine, 14 of 49 brain regions quantified demonstrated significant changes in receptor binding. These regions included the cerebral cortex, caudate putamen, dentate gyrus and dorsal tegmental nucleus. [^{125}I]-BTX receptors were significantly elevated in 23 of 50 regions analyzed. These included the cerebral cortex, globus pallidus, hippocampus, cerebellum and several thalamic and hypothalamic nuclei. Chronic nicotine infusion did not significantly alter muscarinic binding. These data may help elucidate the central mechanisms that regulate nicotine response and the development of tolerance. Supported by DA-03194, MH-16880 and R.J. Reynolds Tobacco Co.

530.5

THE ACTIONS OF ACETYLCHOLINE AND NICOTINE ON NEURONS OF THE VENTRAL TEGMENTAL AREA STUDIED *in vitro*. M.S. Brodie and A.L. Mueller, Neuroscience Research Division, Pharmaceutical Discovery, Depts. 47W and 47H, Abbott Laboratories, Abbott Park, IL 60064.

The ventral tegmental area (VTA) is a brain region which has been demonstrated to mediate at least a portion of the rewarding properties of a wide variety of drugs of abuse. Nicotine is one of the most prevalent agents of abuse in American society. While the effects of nicotine on the neurons of the VTA have been studied *in vivo*, these studies have been restricted by the limitations of *in vivo* recording techniques. We have developed a brain slice preparation of the VTA in order to study the effects of known concentrations of agents on neurons in this area. Brain slices containing the VTA were prepared from Sprague-Dawley rats, and were superfused at 2 ml/min. The cells that were studied with intracellular and extracellular recording exhibited electrophysiological properties similar those of dopamine-containing cells recorded *in vivo* and *in vitro*. Acetylcholine (ACh, 1-10 mM) and carbachol (1-10 μM) produced large increases in the spontaneous firing rate of neurons of the VTA. Addition of physostigmine decreased the effective concentration range of ACh to 30-300 μM. Nicotine (1-10 μM) produced depolarization and an increase in firing rate. Nicotine-induced excitation was completely blocked by the addition of hexamethonium (HEX, 500 μM) to the bathing medium. In the presence of 500 μM HEX, ACh still evoked an increase in spontaneous firing rate. This ACh-induced excitation was blocked by the addition of atropine sulfate (100-250 nM) to the superfusate. These data indicate that both nicotinic and muscarinic receptor activation produce excitation of VTA neurons.

530.6

EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF NICOTINE ON BRAIN METABOLISM IN THE RAT. J.Y. Chang, S.A. Azizi, C.D. Myre, and D.J. Woodward *U. of Texas Southwestern Med. Ctr., Dallas, Tx. 75235*

This experiment was performed to investigate the effect of nicotine on uptake of ^{14}C-2-deoxyglucose in rat brain. Groups of rats studied included conditions of halothane anesthesia (.75% in oxygen); quiet-awake but freely moving; and treadmill locomotion (30 sec rest and 30 sec running). The animals treated acutely were injected with nicotine (0.4 mg/kg free base) or saline ten minutes before the infusion of ^{14}C-2-deoxyglucose (150 μCi per kg). Animals were treated chronically by receiving nicotine (0.4 mg/kg free base per day) or saline injections for ten days. Anesthetized rats showed generalized decreases in metabolism but uptake appeared elevated in habenula, fasciculus retroflexus, and interpeduncular nucleus. This pattern was not observed in awake nicotine or saline injected animals. Nicotine caused little effect in anesthetized animals but caused in both awake groups elevated uptake in regions including substantia nigra (pars compacta), superficial gray layer of the superior colliculus and lateral geniculate body, a pattern related to the visual system as described by others. This pattern remained after 10 days of chronic nicotine administration indicating that tolerance did not appear. The influences of nicotine on glucose uptake required awake brain conditions but were not sensitive to the marked differences in behavior between rest and periodic locomotion. *Supported by R.J.Reynolds Tobacco Co. and Biol. Hum. Found.*

530.7

NICOTINIC BINDING IN CHICKEN MIDBRAIN: AUTORADIOGRAPHIC COMPARISON OF ^{125}I-KAPPA-BUNGAROTOXIN, ^{125}I-ALPHA-BUNGAROTOXIN AND ^{3}H-NICOTINE. E.M. Sorenson and V.A. Chiappinelli. Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

We are characterizing the chicken midbrain as a model system for neuronal nicotinic transmission. As a prelude to intracellular electrophysiological studies, we have described the localization of choline acetyltransferase in the chicken midbrain (Sorenson, E.M., et al., Soc. Neurosci. Abstr., 13:1657, 1987). In binding studies, we have observed at least three categories of putative nicotinic receptors using ^{3}H-nicotine, ^{125}I-kappa-bungarotoxin, and ^{125}I-alpha-bungarotoxin (Wolf, K.M., et al., Brain Res., 439:249, 1988). We now compare the autoradiographic localization of these three nicotinic ligands in adjacent transverse sections of the chicken midbrain.

^{125}I-Kappa-bungarotoxin sites appear to be co-localized with the ^{125}I-alpha-bungarotoxin sites. For example, both ligands bind heavily to the nu. semilunaris, the nu. geniculatus lateralis, pars ventralis, and layer f of the stratum griseum et fibrosum superficiale (SGFS) in the optic tectum. Although ^{3}H-nicotine binds to fewer regions than the snake toxins, preliminary results indicate that nuclei which are labelled with ^{3}H-nicotine are also identified by both toxins. In particular, the nu. geniculatus lateralis, pars ventralis, and the layer f of the SGFS contain binding sites for all three ligands.

(Supported by NIH Grant NS17574 to V.A.C.)

530.8

KAPPA-NEUROTOXINS: ESTABLISHMENT OF A NEW FAMILY OF SNAKE VENOM NEUROTOXINS THAT BLOCK NEURONAL NICOTINIC RECEPTORS. V.A. Chiappinelli, K.M. Wolf*, G.A. Grant*[1] and S.-J. Chen*[2]. Dept. of Pharmacology, St. Louis Univ. Sch. Med., St. Louis, MO 63104; [1]Dept. of Biol. Chem., Washington Univ. Sch. Med., St. Louis, MO 63110; [2]Dept. of Biology, Jinan Univ., Guangzhou, China.

We have reported the complete amino acid sequences of two snake venom proteins, kappa-bungarotoxin (Grant, G.A. and Chiappinelli, V.A., Biochem. 24:1532, 1985) and kappa-flavitoxin (Grant, G.A., Frazier, M.W., and Chiappinelli, V.A., Biochem. May, 1988). These neurotoxins demonstrate 82% sequence homology and exhibit similar pharmacological effects, in particular a selective blockade of nicotinic responses in a variety of neuronal preparations (Chiappinelli, V.A., Pharmacol. Therap. 31:1, 1985).

We now report the isolation of two new kappa-neurotoxins from the venom of Bungarus multicinctus. Kappa$_2$-bungarotoxin and kappa$_3$-bungarotoxin exhibit strong sequence homology with kappa-bungarotoxin. Their pharmacological effects confirm their membership in this new class of snake venom neurotoxins. All four kappa-neurotoxins lack a tryptophanyl residue which is invariant in the structurally similar α-neurotoxins. This and several other features distinguish kappa-neurotoxins from α-neurotoxins, and provide clues to the structural differences between the ligand binding site regions of neuronal and muscle nicotinic receptors. (Supported by NSF-INT-8518395 and NIH-NS17574 to V.A.C.)

530.9

ACETYCHOLINE MODULATES RESTING K-CURRENT THROUGH A PERTUSSIS TOXIN-RESISTANT G-PROTEIN IN HIPPOCAMPAL NEURONS. L.D. Brown, S. Nakajima and Y. Nakajima. Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN 47907; Dept. of Pharmacology and Dept. of Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL 60612.

The purpose of the present experiments was to determine if cholinergic excitation produced by a decrease in the resting K-conductance through muscarinic receptors is mediated by a G-protein. We used primary cultures of hippocampal neurons (CA_1 or CA_3) from postnatal (2 to 8-day-old) rats. Application of acetycholine (10 μM) or muscarine (20 μM) by pressure ejection produced an inward current (peak 50-100 pA) at -74 mV under whole cell voltage clamp. This inward current was inhibited by atropine (1-2 μM). When the internal solution contained GTP (100 μM), this inward current slowly subsided, the recovery being 71% at 3 minutes. However when the internal solution contained GTPγS (200 μM), a hydrolysis-resistant GTP analogue, the inward current tended to persist, the recovery being only 41%. This insufficient recovery with GTPγS was more pronounced for the cells that showed a slow start of the inward current. Pretreatment of cultures with pertussis toxin (500 ng/ml) overnight did not affect the responsiveness of the neurons toward muscarine. The inward current was not affected by addition of 100 μM cAMP and 1 mM IBMX (an inhibitor of phosphodiesterase) to the internal solution. Thus, the cAMP system is not directly involved in the activation of the inward current. The data suggest that a pertussis toxin-resistant unidentified G-protein is involved in the cholinergic activation of the inward current at resting potential. Supported by a NIH Grant NS 24711.

530.10

REPEATED ADMINISTRATION OF NICOTINE RESULTS IN LONG-TERM DECREASE IN PROLACTIN RELEASE BY ACUTE NICOTINE IN RATS. B.A. Giblin[1]*, M.D. Lumpkin[2], and K.J. Kellar[1]. Depts. of Pharmacology[1] and Physiology and Biophysics[2], Georgetown University Medical Center, Washington DC, 20007.

Nicotine increases plasma prolactin concentrations, which peak 6 to 8 min after injection and return to control levels within 30 min. The nicotine-induced increase in plasma prolactin is dose-dependent, completely blocked by mecamylamine, and appears to be mediated by receptors in the brain. As previously reported by Sharp and Beyer (J. Pharmacol. Exp. Ther. 238:1486, 1986), when nicotine is injected a second time, 60 min after the first injection, the prolactin response is very much diminished or absent. This is consistent with rapid and protracted desensitization of the nicotinic receptors mediating prolactin release following the first injection. When nicotine is injected twice daily (0.8 mg/kg) for 10 days, the prolactin response to an acute injection of nicotine is absent 2, 4, 6, and 8 days after the last of the chronic injections. This apparent inactivation of the plasma prolactin response to nicotine is present when nicotinic receptor recognition sites are increased in the hypothalamus. These results are consistent with the hypothesis that chronic administration of nicotine results in long-term inactivation of nicotinic receptors and that this long-term inactivation, in turn, results in up-regulation of the nicotinic receptor recognition sites.

530.11

AUTORADIOGRAPHIC LOCALIZATION OF NICOTINIC RECEPTORS IN RAT BRAIN USING ^{125}I-NEURONAL BUNGAROTOXIN. D.W. Schulz, E. Aizenman, W.F. White and R.E. Zigmond. Dept. Biol. Chem. and Molec. Pharmacol., Harvard Med. School, Boston, MA 02115.

Neuronal bungarotoxin (NBT) is a peptide that is found in the venom of Bungarus multicinctus along with α-bungarotoxin (BGT). NBT has been shown to block nicotinic synaptic transmission in a variety of neuronal preparations where BGT is without effect. While BGT competes for some ^{125}I-NBT binding, it is thought that functional blockade of nicotinic activity occurs at an NBT-selective site. We recently demonstrated that NBT blocks nicotine-stimulated dopamine release in rat striatum, suggesting that NBT is a nicotinic antagonist in CNS tissue. Therefore, we sought to determine the distribution of ^{125}I-NBT binding sites in the rat brain.

Cryostat sections (20μm) from adult male rat brains were incubated in 2.0 nM ^{125}I-NBT for 1 h at 37°C. Nonspecific binding was determined by adding 1 μM NBT, while some adjacent sections were exposed to either 1 μM BGT or 100 μM nicotine. Areas densely labeled by ^{125}I-NBT include: medial vestibular nuc., nuc. of spinal tract of V, dorsal tegmental nuc., superior colliculus, medial habenula, nuc. of the optic tract, and lateral geniculate nuc. Nicotine competed for binding in these areas, but did not completely block all NBT-selective sites. The distribution of ^{125}I-NBT binding was less widespread than that previously reported for either ^{3}H-nicotine or ^{125}I-BGT (Clarke et al., J. Neurosci. 5:1307). Similarly, ^{125}I-NBT binding was present in many but not all areas where nicotinic receptors have been detected using immunohistochemical methods (Swanson et al., J. Neurosci. 7:3334) and in situ hybridization procedures (Deneris et al., Neuron 1:45). In conclusion, ^{125}I-NBT binds specifically and heterogeneously in rat brain, and a portion of these sites are recognized by nicotine. The regional distribution of ^{125}I-NBT binding differs somewhat from that reported using other methodologies, supporting the hypothesis that there are multiple nicotinic receptor subtypes in rat brain.

530.12

NICOTINE ALTERS DOPAMINE, ACETYLCHOLINE, AND OPIOID PEPTIDE CONTENT IN THE STRIATUM OF THE MOUSE. J.P. Hubble*, K.P. Gudehithlu*, G.A. Tejwani and M. Hadjiconstantinou. Dept. of Pharmacology, The Ohio State Univ. Col. of Med., Columbus, OH 43210.

Nicotine receptors are present in the peripheral and central nervous system. The function of these receptors in the brain is unclear. In vitro studies have shown that nicotine stimulates dopamine (DA) release from striatal slices. We have studied the in vivo effects following nicotine administration on dopaminergic, cholinergic and opioid neurons in the mouse striatum. Dose- and time-response studies were performed to establish the regimen producing the optimal dopaminergic response in the striatum. Based on these results nicotine, 1 mg/kg, s.c., was injected and animals were sacrificed after 60 min in acute experiments. For the chronic studies the same dose of nicotine was injected once daily for 7-14 days. Acute nicotine treatment elevated striatal DA content modestly, and significantly increased homovanillic and 3,4-dihydroxy-phenylacetic acids. The turnover of DA, estimated using alpha-methyl-p-tyrosine, was not altered by acute nicotine treatment. Chronic nicotine administration did not change the striatal DA parameters. Acute administration of nicotine decreased striatal acetylcholine (ACh), beta-endorphin and met-enkephalin content. Chronic nicotine exposure had no effect on striatal ACh, decreased beta-endorphin and increased met-enkephalin content in the striatum. In conclusion, our results provide evidence that nicotinic receptors modulate DA, ACh and opioid neurons in mouse striatum and suggest that, as in the periphery, central nicotinic receptors may participate in complex neuronal interactions.

530.13

DISSOCIATION BETWEEN ADENYLATE CYCLASE INHIBITION AND MUSCARINIC AUTORECEPTOR REGULATION OF [^{3}H]ACETYLCHOLINE RELEASE FROM RAT HIPPOCAMPUS. E.D. Cadman* and T.W. Vickroy. Abbott Laboratories, Neuroscience Research Division, Pharmaceutical Discovery, Abbott Park, IL 60064.

Several lines of evidence indicate that muscarinic receptors (mAChR) are heterogenous. In CNS tissues, activation of M_2 receptors inhibits adenylate cyclase and reduces impulse-dependent acetylcholine (ACh) release from nerve terminals. In the studies described here, we have attempted to ascertain whether inhibition of adenylate cyclase underlies M_2-mediated inhibition of electrically-evoked [^{3}H]ACh release from rat hippocampal slices. The muscarinic agonist carbachol caused a concentration-dependent (EC_{50}=80μM), atropine-sensitive decrease in evoked [^{3}H]ACh release up to a maximum of 80% inhibition. The relative potencies of several muscarinic antagonists in reversing carbachol's effect (atropine, N-methyl atropine > scopolamine >> pirenzepine) were consistent with an M_2-mediated response. In the presence of forskolin (10μM) or 8-bromo-adenosine 3':5'-cyclic monophosphate (300μM), electrically-evoked [^{3}H]ACh release was enhanced by 40-50% but the inhibitory effect of carbachol was unchanged. In addition, pretreatment of slices with N-ethylmaleimide markedly reduced carbachol inhibition of adenylate cyclase in hippocampal membranes but did not significantly alter carbachol inhibition of [^{3}H]ACh release. Taken together, these results indicate that inhibition of adenylate cyclase does not underlie mAChR-mediated inhibition of ACh release.

530.14

M-1 AND M-2 MUSCARINIC MECHANISMS IN THE REGULATION OF STRIATAL ACETYLCHOLINE RELEASE. J.M. Gorell, S. Mostafapour*, J.A. Bueri*. Division of Movement Disorders, Department of Neurology, Henry Ford Hospital, Detroit, MI 48202.

Striatal slices from adult male Sprague-Dawley rats were prelabelled with {^{3}H} choline and superfused in separate experiments with the muscarinic antagonists pirenzepine (PZ;M-1) or gallamine (GAL;M-2) or the agonist oxotremorine (OXO). OXO, 10^{-5} -10^{-8}M, produced a dose-dependent decrease in {^{3}H}acetylcholine (ACh) release, maximally at 10^{-5}M to 44% of simultaneous controls. GAL, 10^{-7}-10^{-5}M, or PZ, 10^{-8} and 10^{-6}M, alone did not significantly affect {^{3}H}ACh release. GAL at 10^{-5}M, cosuperfused with OXO 10^{-6}M, partially reversed OXO's effect to 81% of controls. PZ at 10^{-5}M fully reversed cosuperfused OXO's (10^{-6}M) ACh release-depressant effect.

These results suggest that both M-2 and M-1 muscarinic receptor mechanisms play roles in the presynaptic regulation of striatal ACh release.

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530.15

PARTIAL MUSCARINIC AGONISTS AND SELECTIVE TISSUE RESPONSE. M. Dickerson*, R. Schwarz, L. Coughenour*, H. Tecle* and R.E. Davis. (SPON: W.H. Moos) Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, Mi. 48105.

BM-5, an oxotremorine analogue, has been suggested to be a partial muscarinic agonist with tissue specific agonist or antagonist activity. It is thought to show agonist-like activity in tissues with large receptor reserve and antagonist-like activity in tissues with little receptor reserve. Because of this BM-5 may be useful as a tool in defining tissues with more or less receptor reserve. We have used this approach in studying the agonist- and antagonist-like activity in the peripheral and central nervous system.

BM-5 exhibits partial agonist-like properties in vitro. It possesses high affinity for both muscarinic agonists and antagonist binding sites in the rat cerebral cortex, does not alter K^+-stimulated release of acetylcholine (ACH) but does block agonist-induced decreases in the ACH release from brain slices. These effects are similar to those seen with muscarinic antagonists.

BM-5 has agonist- and antagonist-like properties in vivo. It increases intestinal transit and motility and reverses scopolamine-induced swimming activity much like a muscarinic agonist. However, BM-5 impairs the ability of C57BL/10j mice to perform a water-maze task in a manner similar to muscarinic antagonists.

Thus, the relative agonist/antagonist properties of BM-5 are governed by the response being measured and may be determined by intrinsic receptor reserves of the target tissue. The therapeutic utility of this compound, however, is questionable since it acts as a muscarinic agonist in the periphery inducing unwanted effects and acts as an antagonist in the central nervous system to impair cognitive performance.

530.16

CHANGES IN ^{3}H-QNB AND ^{3}H-GLUTAMATE BINDING IN RAT BRAIN FOLLOWING PERSISTENT SEIZURES INDUCED BY LITHIUM/PILOCARPINE TREATMENT. K. Shahid Salles, G. Friedrich*, M.T. Price and J.W. Olney. Washington University Sch. of Med., St. Louis, MO 63110.

Systemic administration of pilocarpine (30 mg/kg sc) to rats pretreated twenty four hours earlier with lithium chloride (3 meq/kg sc) results in prolonged seizures and acute cytopathological changes in various brain regions. Muscarinic cholinergic receptor activation is presumed to play a role in the initiation of seizure activity since pretreatment with atropine prevents both the seizures and related brain damage. The brain damage resembles that associated with persistent seizures induced by various other methods and is indistinguishable from the excitotoxic type of neuronal degeneration that glutamate (Glu) is known to cause. To further evaluate the relative involvement of glutamergic and cholinergic mechanisms in this seizure-brain damage syndrome, we have studied ^{3}H-QNB and ^{3}H-Glu binding by receptor autoradiography in rat brain at various intervals from 4 hrs to 8 wks after a 3 hr episode of persistent seizures induced by Li/Pilo and terminated by diazepam. Our findings are as follows: both ^{3}H-Glu and ^{3}H-QNB binding were uniformly reduced in early time intervals (up to 2 wks) in nearly every brain region evaluated, including regions that do not display brain damage. The reductions in ^{3}H-Glu and ^{3}H-QNB binding were already evident at 4 hrs and became maximal in the 4hr to 1 wk period then gradually returned toward control levels in the 2-8 wk interval. This pattern was seen in all regions except those known to sustain permanent brain damage; in such regions, binding of both ligands remained severely depressed in the 1-3 wk interval and recovered only moderately in the 4-8 wk interval. In two regions (substantia nigra and nucleus accumbens) a substantial reduction in binding of both ligands at 4-24 hr progressively converted to a significant increase in binding at 4-8 wks. Based on Scatchard analysis, the decrease in binding for both ^{3}H-Glu and ^{3}H-QNB could be accounted for by a reduction in B_{max}. This was accompanied by a decrease in K_D for both ligands. These findings are of limited assistance in clarifying the relative importance of cholinergic vs glutamergic mechanisms in the Li/Pilo seizure-brain damage syndrome since changes in both systems were in the same direction, displayed a similar time course and were of similar magnitude. Supported in part by US Army Contract DAMD17-86-C-6010, ES 00875 and Career Scientist Award MH 38894 (JWO).

530.17

EFFECTS OF REPEATED LOW-LEVEL MICROWAVE EXPOSURE ON CENTRAL CHOLINERGIC RECEPTOR. H. Lai*, M.A. Carino*, A. Horita* and A.W. Guy* (SPON: R.H. Haschka). Dept. of Pharmacology and Psychiatry & Behavioral Sciences, and the Center for Bioengineering, University of Washington School of Medicine, Seattle, WA 98195.

Our previous research (Lai et al., J. Neurochem. 48: 40, 1987; Pharmac. Biochem. Behav. 27:635, 1987) showed that acute (45 min) exposure to circularly polarized, pulsed, 2450-MHz microwaves at power density of 1.0 mW/cm^2 (whole body specific absorption rate of 0.6 W/kg) affected cholinergic activity in the brain of the rat. Sodium-dependent high-affinity choline uptake was decreased in the frontal cortex and hippocampus after the exposure. In the present experiment, rats were exposed in 10 daily 45-min sessions to low-level microwaves, and change in muscarinic receptor, as measured by ^{3}H-QNB binding assay, was studied in the brain at 24 h after the last exposure session. There was a significant increase in the concentration of ^{3}H-QNB binding sites ($p < .005$) in the hippocampus of the microwave-exposed rats as compared to that of sham-exposed controls. No significant change in receptor sites was observed in the frontal cortex, striatum, and hypothalamus.

530.18

INTRACEREBROVENTRICULAR ADMINISTRATION OF HEMICOLINIUM-3 DECREASES URINARY OUTPUT IN THE RAT. G.R. Trimarchi*, A. Germanò*, G.M. Campo* and A.P. Caputi*(SPON:J.J.Buccafusco) Dept. Pharmacology, School of Medicine, University of Messine, I-98122 Messina, Italy.

Activation of brain cholinergic receptors results in a natriuretic and kaliuretic response.This effect seems to be mediated by both nicotinic and muscarinic receptors.Purpose of this study was to determine whether a depletion of brain acetylcholine stores is able to influence diuresis,natriuresis and kaliuresis.Adult male Wistar rats with a chronically implanted intracerebroventricular (ICV)cannula were pretreated with hemicholinium-3 (HC;20ug/10 ul ICV)or saline. At different time after the icv pretreatment the animals received 40ml/Kg saline by gavage and placed in individual metabolic cages.Urine samples were than collected every 2 hours for 6 hours and urinary volume and potassium and sodium concentration were measured.The icv injection of HC produced a time dependent inhibition of the urinary output with the maximal effect occurred using 60-90 min pretreatment time.Furthermore,icv HC produced a significant decrease in potassium (0.42+0.02 vs. 0.30+0.02 mEq/100 g.b.w./6h) and sodium urinary excretion (1.34+0.08 vs. 0.87+ 0.05 mEq/100 g.b.w./6h).

530.19

POSSIBLE INVOLVEMENT OF CALCIUM AND CALMODULIN IN THE REGULATION OF [3H]HEMICHOLINIUM-3 ([3H]HCh-3) BINDING AND HIGH-AFFINITY CHOLINE UPTAKE IN RAT BRAIN. M.D. Saltarelli, C.J. Clingroth, K.Yamada and J.T. Coyle. Department of Neurosciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The sodium-dependent high-affinity choline uptake (SDHACU) system has been regarded as the rate-limiting step in acetylcholine synthesis. Calcium (Ca^{2+}) has been implicated as essential for the activation of SDHACU by many, but not all, investigators. In the present study, we have assessed the involvement of Ca^{2+} and calmodulin in the regulation of SDHACU through the use of the specific binding of [3H]HCh-3, a potent and selective inhibitor of [3H]choline uptake. Potassium depolarization of striatal, cortical, or hippocampal slices resulted in a significant increase in specific [3H]HCh-3 binding when compared to control slices incubated in normal Krebs buffer containing 1.2 mM Ca^{2+}. The changes in specific [3H]HCh-3 binding induced by potassium depolarization in cortical and hippocampal, but not striatal, slices were significantly reduced when Ca^{2+} was removed from the incubation medium. Potassium depolarization-induced activation of [3H]choline uptake in striatal slices was also unaffected by removal of Ca^{2+} from the incubation medium. In addition, a dose-dependent inhibition of potassium-stimulated [3H]HCh-3 binding was observed when striatal slices were incubated in the presence of three inhibitors of calmodulin, trifluoperazine ($IC50 = 20\ \mu M$), W-7 ($IC50 = 40\ \mu M$), and W-5; their potencies for the inhibition of potassium-stimulated activation of [3H]HCh-3 binding are consistent with calmodulin inhibition. Finally, incubation of striatal slices in the presence of the Ca^{2+} ionophore A23187 (20 μM) produced a marked increase in specific [3H]HCh-3 binding, even when Ca^{2+} was removed from the incubation medium. These studies demonstrate regional differences in the regulation of [3H]HCh-3 binding by extracellular Ca^{2+}, and suggest the involvement of calmodulin and intracellular Ca^{2+} in the regulation of SDHACU *in vitro* .

530.20

SPECIFIC ACTIVATION OF [3H]HEMICHOLINIUM-3 ([3H]HCh-3) BINDING IN RAT SYNAPTIC MEMBRANES BY PHOSPHOLIPASE A2. K. Yamada, M. D. Saltarelli, C.J. Clingroth and J.T. Coyle. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We have demonstrated previously that treatment of rat synaptic membranes with phospholipase A_2 (PLA_2) resulted in a significant increase in the specific binding of [3H]HCh-3 as reflected by a 2-fold increase in both the affinity and capacity of [3H]HCh-3 for its binding site on or closely associated with the choline carrier. In the present study, we further characterized the role of PLA_2 upon the activation of [3H]HCh-3 binding. Incubation of rat striatal membranes with phospholipase C failed to produce an increase in the capacity of [3H]HCh-3 binding, though a marked increase in the affinity was observed. Phospholipases B and D failed to produce any changes in the specific binding of [3H]HCh-3 when assayed at pH=7.8. Alternately, PLA_2 treatment resulted in the inhibition of two other neurotransmitter carriers, as indicated by a reduction in the specific binding of [3H]desipramine and [3H]mazindol. The specific binding of [3H]HCh-3 after PLA_2 treatment exhibited an uneven regional distribution which correlated with basal levels observed in untreated membranes. The effects of PLA_2 treatment and potassium depolarization of striatal slices were not additive. Finally, quinacrine, an inhibitor of PLA_2, resulted in a dose-dependent inhibition of the potassium depolarization-induced increase in the [3H]HCh-3 binding in rat striatum, cortex, and hippocampus. These results support the involvement of PLA_2 in the regulation of [3H]HCh-3 binding.

530.21

BIS THIAZOLIUM CATIONS: A NOVEL CLASS OF POTENT CHOLINERGIC AGENTS FOR STUDYING NEUROLOGICAL DISEASE. V.Balasubramanian*, M.D.Saltarelli, J.Coyle and H.Wagner* (Spon: H.Moser). Division of Nuclear Medicine, Departments of Radiology & Neurosciences, Johns Hopkins Medical School, Baltimore, MD 21205.

Although Hemicholinium-3 is a highly selective potent inhibitor of the choline uptake carrier, its inability to cross the blood-brain barrier limits its usefulness for studies in vivo. To circumvent this, we have synthesized and studied several thiazolium cations as potential cholinergic agents. Since these cations can be reduced to the neutral dihydro analogs, we envisioned that they may cross the blood-brain barrier, revert back to parent cations and interact specifically with the cholinergic components. Among these, the bis thiazolium cation derived from 4-methyl-5-(2-hydroxyethyl)-thiazole and 4,4′-bis-bromoacetyl biphenyl is a potent competitive inhibitor of choline uptake (Ki=1nM) as well as [3H]Hemicholinium-3 binding. In contrast bis alkyl or aryl thiazolium cations were less potent ($Ki=10^{-6}$-10^{-7}M). Model studies show that these thiazolium cations can be reduced to the corresponding dihydro analogs which, as expected, showed a propensity to undergo reoxidation to parent cations. These results suggest that bis thiazolium cations may be useful ligands for in vivo visualization of central cholinergic structure and function.

530.22

BINDING OF 3H-HEMICHOLINIUM-3 TO THE HIGH AFFINITY CHOLINE TRANSPORTER IN ELECTRIC ORGAN SYNAPTOSOMAL MEMBRANES. S. O'Regan+(SPON:N. Morel). Dept. Neurochimie, NBCM-CNRS, 91198 Gif-sur-Yvette, France.

Hemicholinium-3(HC-3) is a potent inhibitor of high affinity choline uptake by cholinergic nerve terminals. Indeed, sodium dependent binding of 3H-HC-3 was 10-fold higher with pre- than postsynaptic membranes, and electric organ synaptosomal membranes bound 3H-HC-3 with a $K_D=3 \pm 4$ nM and a Bmax=5.0±0.2 pmoles/mg protein. Choline, HC-3 and their analogues had similar effects on 3H-HC-3 binding and 3H-choline uptake, but there was some evidence of site heterogeneity for 3H-HC-3 binding in the presence of choline. Coexistence of inward and outward facing conformations of the transporter in the membrane preparation may explain this, and the inhibition of 3H-HC-3 binding to intact synaptosomes by choline was monophasic while the Bmax was reduced.

By comparing uptake and binding parameters, an apparent turnover number of 3 sec^{-1} was estimated for the high affinity choline transporter at 20°C under resting conditions. The high transport rates observed with electric organ synaptosomes are likely due to a high density of high affinity choline transporters, thought to be of the order of 100/um^2 synaptosomal membrane on the basis of specific 3H-HC-3 binding.

530.23

CARBAMYL-APROPHEN ANALOGS FUNCTION AS BOTH ANTIMUSCARINICS AND ANTICHOLINESTERASES. R.M. Smejkal*, H. Leader*, C.S. Payne*, A.D. Wolfe*, N.D. Brown*, R.K. Gordon*, and P.K. Chiang. Walter Reed Army Institute of Research, Washington, DC 20307-5100.

A series of aprophen analogs were synthesized with carbamyl substitutions at the para position of the phenyl rings to determine whether such compounds would display binary drug functions with therapeutic characteristics of aprophen as well as the ability to mask cholinesterases. These compounds carbamylate human butyrylcholinesterase with K_I values similar to those of pyridostigmine and physostigmine. The antimuscarinic profile of the analog containing a monomethylcarbamate moiety at the para-position of one phenyl ring of aprophen was very similar to that of aprophen in terms of its inhibitory effect on the acetylcholine-induced contraction of guinea pig ileum, the carbachol-induced release of α-amylase from pancreatic acinar cells and the binding of [3H]NMS to bovine brain membrane. The dimethylcarbamyl-substituted analog was about as active in inhibiting ileum contraction, but the inhibition of α-amylase release and [3H]NMS binding was one order of magnitude lower. Substitution on both phenyl rings was not well tolerated. These data suggest that one of the phenyl rings of aprophen must remain unsubstituted in order to preserve antimuscarinic activity, while bulky substitutions on the phenyl rings can be tolerated by butyrylcholinesterase.

530.24

THE EFFECT OF AGING ON MUSCARINIC-STIMULATED PHOSPHATIDYLINOSITOL (PI) TURNOVER IN FISHER 344 RATS. R.D. Schwarz, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Receptor number and function of central neurotransmitters, including ACh, have been shown to be altered during aging and in neurodegenerative disorders associated with aging (e.g. Alzheimer's disease). Recently, we reported that cortical M1 muscarinic receptors were significantly decreased in aged Fisher 344 rats (J. Cell. Biochem. 11D: 198, 1987). Since it has been suggested that M1 receptors are functionally coupled to phosphoinositide hydrolysis, the present study examined whether there are age-related changes in PI turnover. Using the method of Berridge et al., 1982 the effect of various muscarinic agonists and antagonists on PI turnover was measured in cortical slices from young (3-5mo) and aged (22-24mo) male Fisher 344 rats. In young rats carbachol produced a concentration-dependent increase in total [3H]-inositol phosphates which was blocked by scopolamine with other agonists behaving as partial agonists. In aged rats, similar effects were observed. There were no significant differences between the two age groups comparing dose-response curves and maximal responses, however, the results were more variable. Thus, the previously observed decrease in cortical M1 receptors of aged Fisher 344 rats does not appear to be functionally translated into altered PI turnover.